THE UNIVERSITY OF ALBERTA

A STUDY OF DIFFERENTIATION AND MATURATION IN WHITE MUSTARD ROOT TIPS

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled A STUDY OF DIFFERENTIATION AND MATURATION IN WHITE MUSTARD ROOT TIPS submitted by Robert Lawrence Peterson in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Detailed measurements of levels of tissue differentiation and maturation were made on both fresh and permanent sections of white mustard root tips grown in a moist-air environment at room temperature. Results clearly showed that each tissue differentiates and matures at its own rate. Definite regions in which one developmental process dominates could not be recognized in these roots.

A comparison of levels of differentiation and maturation of primary tissues in root tips grown in moist-air environments, aerated water culture solutions, and non-aerated water culture solutions showed the range of variation in the tissues studied. In general, the three environments seemed to have little effect on the levels of development of primary tissues with the exception of the appearance of root hairs and the commencement of lignification and maturation of protoxylem.

The work on intercellular spaces included the following: the measurement of intercellular space size from prepared cross sections of roots grown under atmospheric and reduced pressure; a series of experiments undertaken to determine whether the spaces are empty or contain a gas, liquid or solid, or all three; and a series of microchemical tests to determine the chemical nature of any one of these substances. Microchemical tests included well-known chemical reactions for proteins, lipids, callose, and carbohydrates. At a

level close to the root apex, some of the spaces appeared to contain a "solid-like substance". Other spaces which lacked this substance gave a positive test for a reducing sugar. Subsequent testing of the "solid-like substance" indicated the presence of lipids. Spaces in the mature regions of the root always appeared empty when viewed in transverse section.

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INTRODUCTION

Although the literature on the processes of differentiation and maturation in growing root tips is voluminous, much of the evidence is conflicting and there is increasing need for a revision of classical terms and ideas in the light of modern investigations. Early workers described root-tip development in terms of distinct regions in which one morphogenetic process predominated. The terms "zone of cell division", "zone of elongation", "zone of differentiation", and "zone of maturation" were adopted and are perpetuated in present-day textbooks. However, evidence in opposition to this classical conception of root-tip development is slowly accumulating. In his discussion of differentiation and maturation, Popham (1955) states, "If the truths of tissue differentiation and maturation are too complex to be taught to students of botany, misconceptions of a bygone era are not valid substitutes." It seems probable that a general pattern for the development of the root tip can be formulated and the range of variation from the general pattern anticipated if a sufficient number of species is examined in detail.

Popham (1955), called attention to the confusion in the literature concerning the term "differentiate". He states, "It is hazardous and frequently impossible to compare published data concerning levels of tissue differentiation because this term is so inaccurately and inconsistently used ...". Cells

or tissues can be considered differentiated if they are different physiologically or morphologically enough to become easily recognizable. In the present study, only initial morphological differences indicative of either differentiation or maturation are considered, and for each cell or tissue the criterion or criteria used will be indicated.

The intercellular space system of the plant has been studied by many workers, and from the standpoint of tissue development and nutrition, has aroused much interest.

The present study is an attempt to add to our knowledge of differentiation and maturation in growing root tips by further microscopical and microchemical studies. For convenience, the subject matter is divided into three sections:

A. Development of the root under normal conditions.
B. Experimental (effects of environment on root development).
C. Intercellular spaces and their content.

REVIEW OF LITERATURE

A. Tissue Differentiation and Maturation Under Normal Conditions

The tips of growing roots are commonly used to illustrate the principal steps in tissue differentiation: cell division, cell elongation, and cell maturation (*e.g.* Fuller *et al.*, 1957; Robbins *et al.*, 1957; Wilson *et al.*, 1962), but the demarcation between regions in which each of these processes occurs exclusively can be made only approximately, because at any one level in the root two or three processes may overlap not only in different tissue regions but within the same region (Esau, 1953b; Goodwin and Stepka, 1945; Popham, 1955; Thompson, 1960).

The regions of the root tip, the epidermis, root cap, cortex and vascular cylinder, are delimited close to the apical meristem but differentiation and subsequent maturation of component tissues of these regions proceed at different rates.

Root epidermal cells of some plant species, including white mustard, show a marked difference in size and ability to form root hairs. This observation has led to much research in an attempt to explain the variation in differentiation of genetically identical sister cells.

A thorough study of *Phleum pratense* primary roots has revealed a difference in growth rate of the trichoblasts (the shorter cells producing the root hairs) and the longer hairless cells (Avers, 1957; Avers and Goodwin, 1956; Brumfield,

1942; Goodwin and Avers, 1956; Sinnott, 1939; Sinnott and Bloch, 1939a, 1939b). The difference in elongation rates of trichoblasts and hairless cells occurs only within a short region of the root apex, just basal to the apical meristem (Avers, 1957). Trichoblasts and hairless cells increase in length at the same rate in the meristem and in regions basal to this region. This observation suggests that physiological differentiation of the two cell types probably occurs in the area of observed differences in growth rate. Histochemical tests were then conducted for differential enzyme activity in the epidermal cells (Avers, 1958; Avers and Grimm, 1959a). The most intense activity of the enzymes acid phosphatase and cytochrome oxidase was localized in young trichoblasts during their early elongation phase. Succinic dehydrogenase activity was localized in the meristematic tissue, the future hairless cells showing no activity and the trichoblasts showing a high activity. Observations on other grass species (Avers and Grimm, 1959b), showed that although the time of physiological differentiation of the epidermal cells varies with the species, a correlation between the pattern of epidermal development and the presence of acid phosphatase existed in all cases. Further observations upon the differential localization of these and other enzymes (Avers and Grimm, 1959b; Czernik and Avers, 1964), and the heterogeneity of cellular organelles (Avers, 1961; Avers and King, 1960), strengthened this cytochemical view of tissue

differentiation.

In the region of epidermal cell differentiation in Phleum pratense i.e. 100-300 microns from the root apex (Avers, 1957), both symmetrical and asymmetrical mitoses occur, the latter resulting in a smaller apical cell which develops a root hair at maturity and a larger basal daughter cell which remains hairless (Avers, 1963). An electron-microscopic comparison of cells showing symmetrical mitosis with those showing asymmetrical mitosis (Avers, 1963), clearly showed that a greater number of cytoplasmic organelles moved to the basal end of the asymmetric cell. Since this is directly opposite to what is expected on the basis of histochemical tests, the author interprets the ultrastructure variation as "reflections of mitotic events which are not immediately related to the different physiologies of the daughter cell products after cytokinesis."

In white mustard, the dissimilar epidermal cells show a marked difference in vacuolation rates (Cormack, 1947). A possible explanation for this difference has been developed in a series of papers (Cormack, 1935, 1947, 1948; Cormack and Lemay, 1963). The identification of a substance capable of reducing alkaline silver in the intercellular spaces of this root (Cormack, 1948), the detection of intercellular inclusions induced by colchicine treatment (Cormack, 1949), and the evidence that sugar can be translocated from the cotyledons to the apical meristem by the intercellular space system

(Cormack and Lemay, 1963), supports the early explanation of the difference in vacuolation rates of the epidermal cells in this root. *i.e.* ... "if the intercellular spaces are at first filled with a nutrient sap and the intervening walls relatively impermeable, then a short cell in contact with a small intercellular space of its own and in direct line with a series of larger spaces in the cortex would appear to be in a much more advantageous position for obtaining nutrients than its neighboring long cells" (Cormack, 1947).

Root-hair development is intimately connected with the condition of the epidermis. In roots with both short and long epidermal cells, the former normally develop hairs while the long cells remain hairless. The mechanism of root-hair development and the factors affecting this process have been recently reviewed (Cormack, 1949, 1962).

In many roots, the root cap and the epidermis have a common origin in the apical initials. The structure and function of the root cap has received much attention in the literature (*e.g.* de Bary, 1884; Eames and MacDaniels, 1947; Esau, 1953a; Strasburger, 1921). The root cap is generally mature closer to the apex than any other tissue and can be recognized as the only vacuolated cells in transverse sections immediately adjacent to the meristem (Clowes, 1961). Starch, thought to be used only in cases of extreme starvation (Netolitzky, 1935), is often present in the root-cap cells (de Bary, 1884; Esau, 1940, 1953a). Histochemical

staining procedures have shown a correspondingly high phosphorylase activity in this region of the root (Dyar, 1950; Yin and Sun, 1947, 1949). Richardson (1955), has shown that some roots normally growing in soil may lack root caps when grown in water culture. During the course of an investigation on the development of root hairs by tomato roots, Cormack (1945) made an interesting observation on the root caps of many roots growing in alkaline calcium solutions. Root-cap cells were found adhering to the epidermis for a considerable distance from the apex, and in some roots the cells were found "hanging together" and curled over the extreme tip of the root.

Measurements to show the extent of the root-cap tissue apically from the meristem have been recorded for some plants (Popham, 1955; Torrey, 1955), and the persistence of root-cap tissue basally from the initials has been indicated for others (Cormack, 1947; Thompson, 1960).

Thorough microscopical observations have been made on root cortical cells. Cortical tissue of the primary root consists exclusively or chiefly of parenchyma, the orderly arrangement of cells resulting from the method of cell division during the origin of this region (de Bary, 1884; Esau, 1953a). Repeated periclinal divisions in the inner row of cortical cells increases the diameter of the cortex within a short longitudinal distance from the root apex (Heimsch, 1960; Williams, 1947). From the standpoint of development, the outer cortical cells are older than the inner and

consequently at any one level appear larger in transverse section. At intervals, divisions in the outer cortex occur which allow for increase in diameter during root growth. Once periclinal divisions have ceased in the inner row of cells, a Casparian strip is laid down on the radial and transverse walls, typifying this cell row as the endodermis (Esau, 1940, 1941, 1953a; Popham, 1955; Williams, 1947). Vacuolation of cortical cells generally begins in the middle cortical rows as these cells are the first to stop dividing, and proceeds both centrifugally and centripetally (Clowes, 1961; Popham, 1955). Cortical tissue may persist throughout the growing period of short-lived roots but in roots that develop a great deal of secondary tissue, this region becomes crushed and is sloughed along with the epidermis.

Although the development of the primary vascular tissues has received the greatest attention, detailed information comparing the differentiation and maturation rates of one vascular tissue with another is relatively meagre and widely scattered. Primary vascular tissue in the root originates from a group of meristematic cells which have been formed by the apical initials. This region has been variously interpreted and named in the literature (see reviews by Esau, 1943b, 1954), but generally the term "procambium" has been accepted. Early investigations (Esau, 1943b), as well as later studies on initial vascularization in roots (Bunning, 1951; Esau, 1943a; Goodwin and Stepka, 1945; Hayat and Heimsch, 1963; Heimsch, 1951; Popham, 1955; Torrey, 1953, 1955), have

led to the following conclusions. Vascular elements differentiate acropetally. Metaxylem cells are recognizable closer to the apical initials than any other vascular tissue because of their early enlargement and vacuolation. Bunning (1951), attributes the diarch xylem pattern in Sinapis alba L. to the polarity of the first-differentiated metaxylem cell. This author claims that later primary xylem cells always differentiate along an extension of the radial polarity axis of this first cell. Protoxylem cells mature earlier than metaxylem and beginning at the periphery of the xylem plate, maturation proceeds centripetally. The first phloem cells differentiate after the first xylem but mature closer to the apical initials.

B. Tissue Differentiation and Maturation in Experimental Environments

The relation of the growth rate to the differentiation pattern and the extent to which this relation can be modified are critical in many fields of research (Heimsch, 1951), yet few comprehensive studies of this nature have been undertaken. Bryant (1934) and Popham (1955), compared some anatomical aspects of primary tissue development in roots grown in aerated and non-aerated culture solutions. In both studies, root length attained in the experimental period was greatest in the aerated solutions. Bryant found lignified xylem elements and a well-developed pericycle and endodermis

further from the apex in these roots than in those grown in the non-aerated solutions. Popham reached the general conclusion that levels of tissue differentiation and maturation of all primary tissues in the root of Pisum sativum were closer to the apex in the non-aerated solutions, but in many cases the differences were only slight.

Heimsch (1951), studied the effects of growth rate on vascular tissue differentiation in barley roots. Although the distance from the apex to the first differentiated phloem and xylem varied little with growth rate, considerable variation occurred in the maturation of these same tissues. Generally, in slow-growing roots the distance is shorter than in fast-growing roots. Vascular tissue of many roots show this same pattern of development (Barghoorn, 1942; Esau, 1943b, 1954).

C. Intercellular Spaces and their Content

Two comprehensive reviews (Sifton, 1945, 1957) include the major studies on intercellular spaces. The origin of the schizogenous spaces that are common to most root cortical tissue can be summarized briefly as follows. When a cell divides, the new middle lamella is separated from the old one of the original cell by a layer of cellulose wall. The free ends of the new middle lamella then thicken so that in transverse section a triangular mass is seen. This mass enlarges and becomes a cavity. As the cavity grows, the cellulose wall between it and the middle lamella of the

mother cell dissolves and the intra-wall cavity becomes an intercellular space. As the cells grow and pull apart from each other along extended areas of their walls, these spaces become larger.

The intercellular material lining the newly-formed spaces was interpreted by some early workers as cytoplasm, while others held that it was of a "pectic nature". Recently the composition of this lining has received more attention, and from infiltration experiments and microchemical tests, protein, cutin, and suberin have been suggested as possible constituents (Scott, 1948, 1950, 1963; Scott _et_ _al_., 1953; Sifton, 1957).

The content of intercellular spaces has been a matter of much conjecture and still remains a controversial issue. Priestley (1929), was the first to suggest the presence of a nutritive liquid in the newly-formed spaces and attributed the ability of the apical cells to remain meristematic to the movement of a "nutritive sap" in the spaces and along the carbohydrate cell walls. Early workers generally agreed that the spaces serve as an "aerating mechanism" for the internal tissues of the plant (Sifton, 1945). In an anatomical comparison of roots of white mustard and tomato (Cormack, 1947), the probability that soluble nutrients may be transported by the intercellular space system was again suggested. Experimental evidence strengthened this view (Cormack, 1948; Cormack and Lemay, 1963).

A recent publication, (Sorokin, 1958), shows clearly the presence of an "intercellular tubular material" in the growing regions of pea stems in addition to the usual intercellular lining. According to this author, this intercellular substance is composed of an outer lipid membrane, an unidentified inner "gray matter" and a central lumen for air or gas.

The influence of intercellular spaces on the differentiation and maturation of growing tissues has received only limited attention in the literature. Lewis (1923, 1933), although recognizing the presence of such spaces in the various tissues used for cell shape determinations, disregarded their influence because of their small size. Later workers (Flint, 1949, 1951; Higinbotham, 1942; Hulbary, 1944), found a significant reduction in the number of cell faces in undifferentiated tissues if intercellular spaces were taken into consideration. On the basis of Scott's work (Scott, 1948, 1950; Scott *et al.*, 1953), Sifton (1957) suggests that if the lining of the intercellular spaces is composed of polymerized fats, it may through its rigidity be a factor in determining the final size and shape of cells and intercellular spaces.

Sinnott and Bloch (1946), attributed the formation of a trichosclereid from the smaller of a pair of daughter cells in the cortex of the air roots of *Monstera deliciosa* to the proximity of the smaller cell to the intercellular space

system. Similar cells in the hypodermis fail to develop trichosclereids due to the absence of intercellular spaces in this tissue. As already noted, the intercellular space system in the roots of white mustard is held to be an important factor in prolonging the protoplasmic condition in the trichoblasts of the epidermis (Cormack, 1947, 1948; Cormack and Lemay, 1963).

MATERIALS AND METHODS

PLANT MATERIAL

White mustard (Sinapis alba L.) was chosen for this study since previous anatomical observations (Bunning, 1951; Clowes, 1961; Cormack, 1935, 1947, 1948), provided basic information on tissue origin and organization in the primary root. However, a detailed anatomical study involving the differentiation and maturation rates of all primary tissues has not been reported.

METHODS

A. Tissue Differentiation and Maturation under Normal Conditions

1. Standard growth procedure

Seeds of Sinapis alba L. obtained from Steele-Briggs Seed Co., Edmonton, were germinated and grown on filter paper moistened with distilled water. Petri dishes containing the seeds were placed in the dark at room temperature for a growing period of 24 hours after germination, by which time the primary root had reached 1.0-2.5 cm. in length and had developed numerous fine hairs. The length of 20 randomly selected roots was determined to the nearest 0.5 mm. with a fine ruler (Appendix I) and those to be used for permanent slides were severed below the hypocotyl with a sharp razor blade.

2. Preparation of permanent slides

Excised root tips were fixed in CRAF V (Sass, 1940) for

periods up to 24 hours. Dehydration of the roots with a tertiary butyl alcohol series beginning with 50% and ending with absolute alcohol in preparation for embedding was carried out. A small amount of Erythrosin B which stained the roots lightly, to aid in locating them once they were embedded, was added to the vials containing the 100% alcohol. A simplification of the usual embedding technique (Johansen, 1940), was developed and found to be very satisfactory. After the roots had been carried to absolute butyl alcohol, they were placed directly into vials containing used Fisher Tissuemat (m.p. 56.5° C.). Here they were left for 24 hours or until the last trace of butyl alcohol had disappeared. A further change of used Tissuemat, followed within 8 hours by two successive changes of unused Tissuemat, completed the embedding procedure. The hot Tissuemat containing the roots was poured quickly into 2 X 6 cm. paper boats on a warming plate, the roots arranged with a warm needle, and the Tissuemat solidified uniformly by submerging the boats in ice water. The embedded roots were mounted on wooden blocks and the excess Tissuemat trimmed with a razor blade.

To follow the developmental changes in the roots, both transverse and longitudinal sections were cut on a rotary microtome (American Optical Company, Model 815). Transverse sections were cut at a thickness of 12 microns beginning at the extreme apex of the root while longitudinal sections were cut at 8-10 microns. All sections were affixed to

microscope slides with Haupt's adhesive (Johansen, 1940). For most sections, a staining procedure according to the following scheme was used.

a) Absolute xylene
b) 50/50 absolute xylene-absolute ethanol
c) 95% ethanol
d) 85% ethanol
e) 70% ethanol
f) Safranin in 70% ethanol
g) 85% ethanol
h) 95% ethanol
i) Fast Green in 95% ethanol
j) Absolute ethanol
k) 50/50 absolute xylene-absolute ethanol
l) Absolute xylene

On occassion, Delafield's Haematoxylin or Crystal Violet was substituted for Safranin and Fast Green and the procedure as outlined by Johansen (1940) was followed. The stained tissue was mounted in Permount (Fisher Scientific Company).

3. Photography

All photomicrographs were taken with a Zeiss Photomicroscope (55575) using Kodak Panatomic-X Film (FX 135-20). Films were developed in a 1:1 Dektol (Eastman Kodak Co.)-tap water solution at 68° F., rinsed for 20 minutes in cool flowing tap water, immersed in Photo-flo (Eastman Kodak

Co.) for 30 seconds and hung to dry.

Prints on Kodak Kodabromide single weight F-4 paper (Canadian Kodak Co.) were developed in the same manner as the films. All photomicrographs were enlarged with a Beseler Enlarger (Model 45MX), as specified for each figure in the paper.

B. Tissue Differentiation and Maturation in Experimental Environments

1. Aeration

Seeds that had been germinated in the usual manner were placed on waxed filter paper floats with their newly-emerged radicles submerged in the culture solution. Since the experimental period was very short, the culturing medium was simply distilled water. In one set of experiments, the floats bearing the seedlings were placed on the distilled water in the dark at room temperature and allowed to grow for 24 hours. To study the effects of aeration on tissue differentiation and maturation, the second set of experiments differed only in that a fine stream of pure oxygen was bubbled through the distilled water for one-half hour before and during the experimental period.

2. Temperature

Experimental conditions for the third category of roots were nearly identical to those used for the growth of roots for the major part of this study. Seeds were germinated on

moist filter paper in a petri dish in the dark at room temperature and then transferred to a constant temperature chamber. A range of temperatures (37° F. - 100° F.) were tried to determine the effect of temperature on root-tip development.

The length, to the nearest 0.5 mm., of 20 randomly selected roots from each experimental category was obtained (Appendix I), and all roots selected for sectioning were fixed and embedded as before.

C. Intercellular Spaces and their Content

1. Space size

A comparative study of roots grown under reduced pressure with control roots grown under atmospheric pressure was undertaken to investigate the content of the intercellular spaces. This experiment was undertaken on the assumption that if the intercellular spaces contain nothing but a gas, an increase in size or a misshaping of the spaces might result, due to the expansion of this gas against the thin meristematic cell walls.

For growth of roots under reduced pressure, seedlings were grown in a specially designed apparatus. Seeds that had just germinated were placed on moist filter paper in the bottom of a small narrow-mouthed jar. A rubber balloon containing enough commercial oxygen to just inflate it was placed over the mouth and the jar made air-tight with string

and hot paraffin. The jar was then placed inside a vacuum dessicator connected through a manometer to a Duo-seal vacuum pump. Air was removed from the dessicator until a pressure of 5-10 mm. of mercury was recorded on the manometer. In so doing, the balloon expanded to occupy the greater volume of the dessicator, resulting in a decreased pressure around the roots. The experiment was continued until the roots had reached 1-2 cm. in length. Permanent slides were prepared in the usual manner, in preparation for examination and measurement of the intercellular spaces.

2. Intercellular space content

Fresh sections of primary roots grown on moist filter paper were used for investigations of intercellular space content. For most of the histochemical and solubility tests, additional fresh sections of one or more of Ricinus communis variety Zanzibarensis, Pisum sativum, or Zea mays grown in a similar way were used to confirm observations made on white mustard. All fresh sections were cut directly into distilled water or other experimental media in preparation for examination under the microscope. Detailed methods and concentrations of dyes and chemical reagents will be given with the results.

RESULTS

A. Tissue Differentiation and Maturation under Normal Conditions

Although a detailed study of tissue origin was not intended in this study, median longitudinal sections prepared for confirmation of other data, show the organization of the apex rather clearly. Various interpretations of the promeristem of this root have been diagrammed and discussed in a recent text (Clowes, 1961). In the present study, the structure of the differentiating root tip is analyzed in terms of the mature regions to be formed; the epidermis and root cap, the cortex, and the vascular cylinder (after Esau, 1953a). Fig. 1 shows the three initial layers which give rise to the epidermis-root cap, the cortex, and the vascular cylinder, as outlined in fig. 2.

The data obtained from examination of roots grown under normal conditions (a moist-air environment at room temperature), provide basic information of the appearance and levels of differentiation and maturation of primary tissues in the root tip of white mustard. The levels at which differentiation and maturation of tissues take place are given in terms of distance behind the root-cap initials (see figs. 1, 2 and 4). For convenience this region will be referred to as the root apex or apex. The distances behind the root apex were calculated by multiplying the number of sections removed from the apex by the thickness of the section (12 μ's).

The results are presented for each individual tissue,

PLATE I.

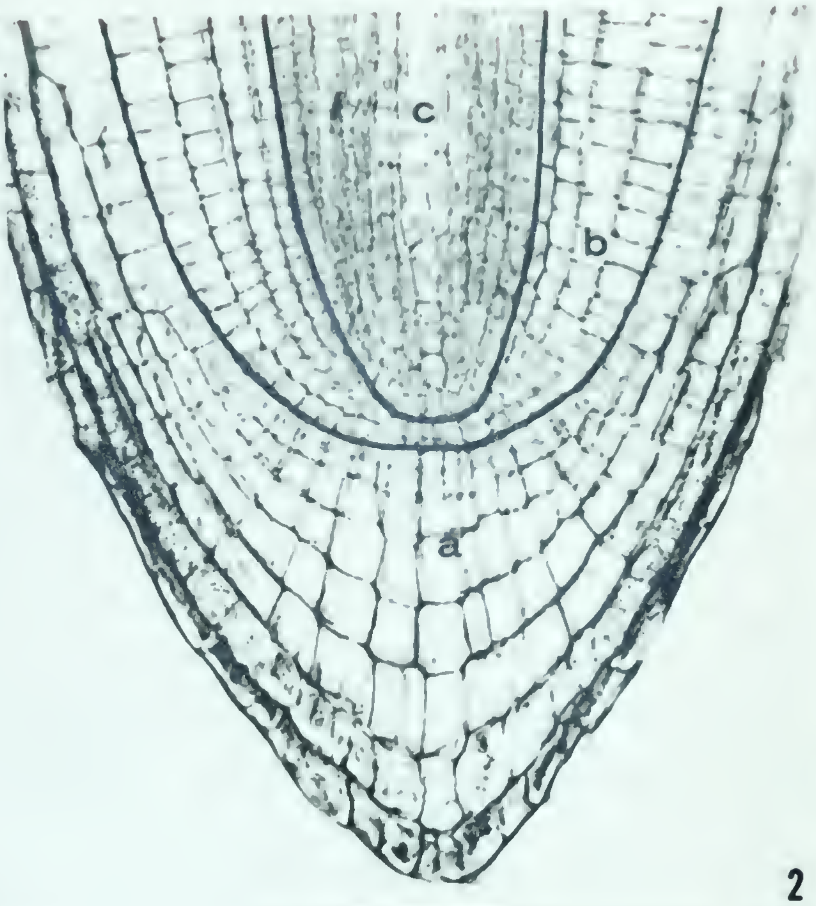
Figure 1. (X280) Median longitudinal section of white mustard root tip showing the three layers of initial cells in the apical meristem. (a) initials which give rise to the epidermis-root cap (b) initials which give rise to the cortex (c) initials which give rise to the tissues of the vascular cylinder.

Figure 2. (X280) Median longitudinal section of the root tip showing the three primary tissue regions derived from the initials marked in Figure 1. (a) epidermis-root cap (b) cortex (c) vascular cylinder

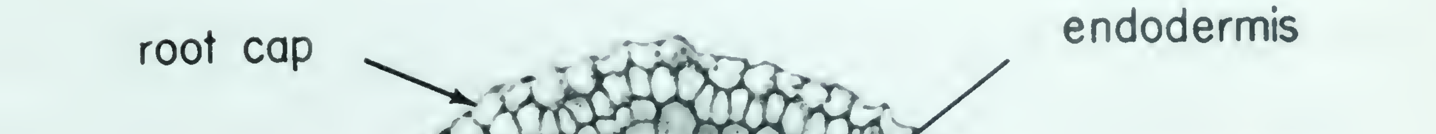
Figure 3. (X220) Transverse section of the root tip about 300 μ's from the root apex showing organization of primary tissues.


c
b
a

1


c
b
a

2


root cap
epidermis
short cell
long cell
cortex
endodermis
pericycle
phloem
xylem
vascular cylinder

3

followed by a summary to show the pattern and spatial relationship of tissue differentiation and maturation in this root. Fig. 3 shows the typical arrangement of tissues as seen in transverse view at the level of early differentiation in the vascular cylinder, and should be referred to for an understanding of tissue organization.

1. Root cap

The main function attributed to the root cap is that of protecting the delicate meristematic cells during growth of the root through the soil. The root cap consists of a cone-shaped mass of cells which are constantly being replaced by initials in the apical meristem. The depth of the root-cap cone was determined from measurements of prepared median longitudinal sections by means of a calibrated eyepiece micrometer (see Table I and fig. 28). In the roots of white mustard, as in most dicotyledonous roots, part of the root cap lies over the surface of the rest of the root because some of the cells at the periphery of the cap differentiate on the basal side of the initials and some on the apical side (figs. 1 and 2). Root-cap cells vacuolate rapidly, and as a result are the only vacuolated cells to be seen in transverse sections at a level slightly above that of their initiation (figs. 4 and 5). By studying transverse sections, the persistence of the root-cap tissue surrounding the rest of the apex was determined (Table I). Figs. 5-9 clearly show the gradual decrease in thickness of the root-cap tissue with increase in distance from the root apex.

PLATE II.

Transverse sections of the root tip of white mustard showing some of the obvious changes in the primary tissues at various distances back from the apex.

(all X180)

Figure 4. Section through the root-cap tissue immediately preceding the apical initials. This section is referred to as the root apex or apex throughout the present study.

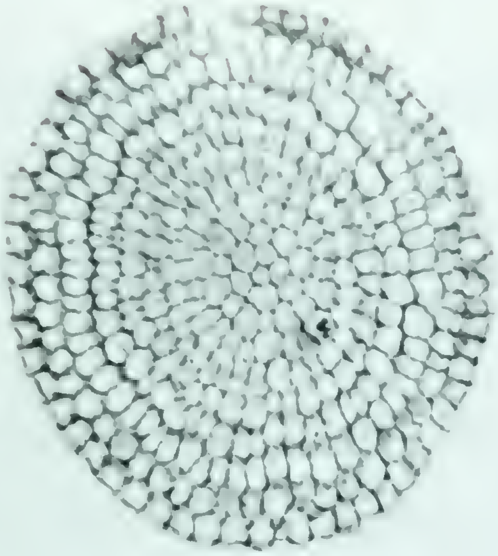
Figure 5. Appearance of the root tip approximately 80 µ's from the root apex. The differentiated epidermal cells surrounded by root-cap tissue, the five rows of cortical cells inclusive of the endodermis, the single row of pericyclic cells, and the narrow vascular cylinder with its diarch xylem plate already formed, are obvious. At this level, small intercellular spaces appear in the cortex.

Figure 6. The same root 150 µ's from the apex. The root-cap tissue has decreased slightly in thickness, and an increase in circumference of the root is obvious.

Figure 7. At 300 µ's from the apex, the root-cap tissue has diminished still further and the root has increased in diameter due mainly to cell enlargement. The vascular cylinder has increased in size and one sieve tube has differentiated.

Figure 8. At 600 µ's from the apex, vacuolation of the long cells of the epidermis and of some of the cortical cells is evident. The root-cap tissue surrounding the rest of the root is now two cell layers thick and in many places the outer layer of cells is becoming crushed. The cortical intercellular spaces have continued to enlarge and are easily visible. Within the vascular cylinder, two sieve tubes (the cells lacking any contents) have become mature.

Figure 9. Section of the root tip at approximately the 850 µ level. The long epidermal cells have become conspicuously vacuolated while the short cells remain protoplasmic. The outer cortical cells have also become vacuolated. At this level, the root-cap tissue is restricted to a single layer of cells surrounding the root.



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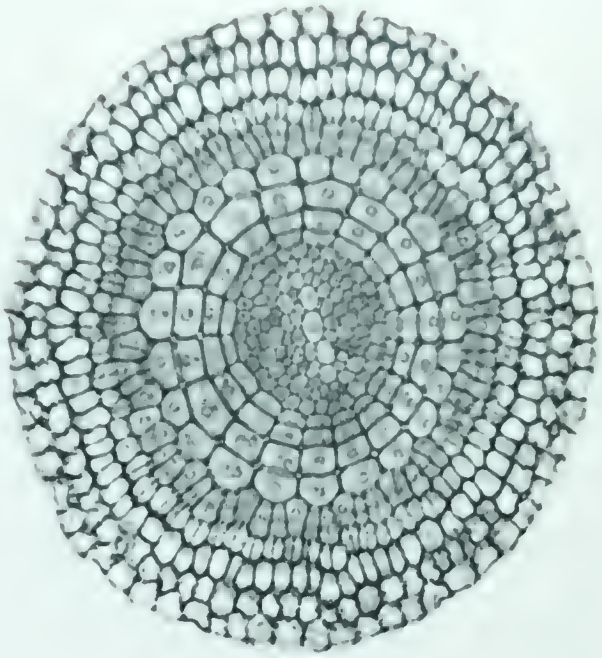

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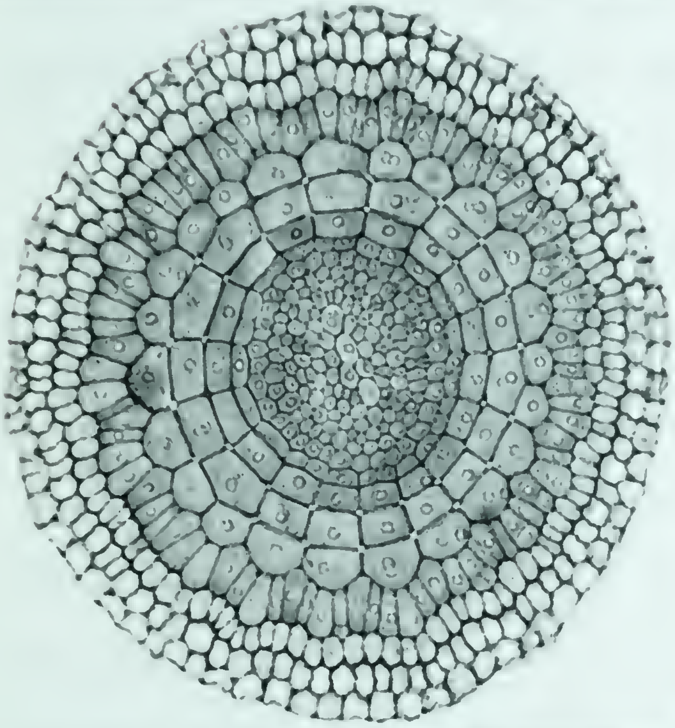

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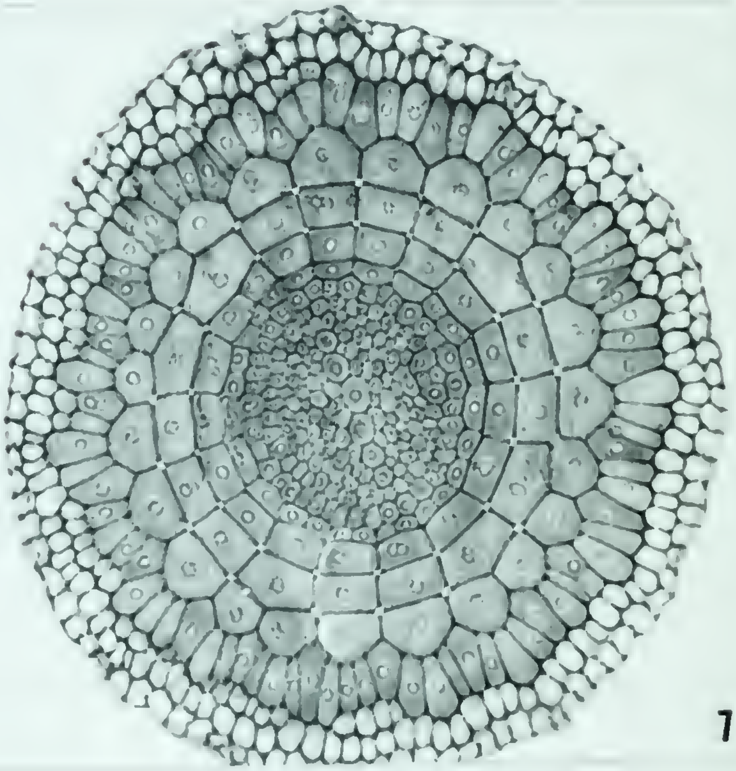

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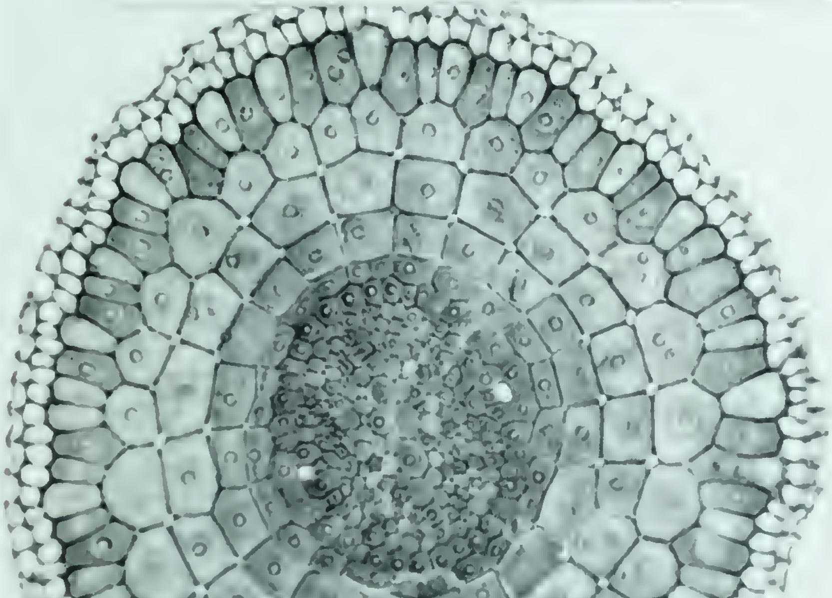

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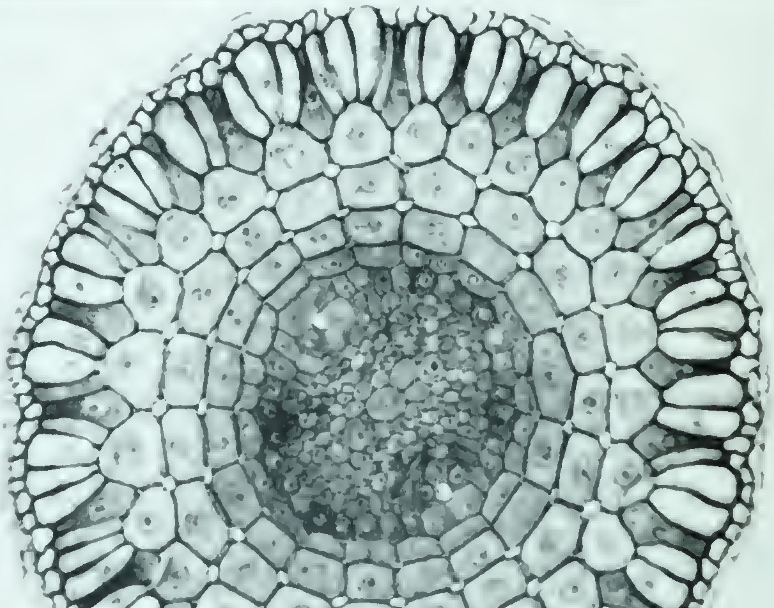

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One complete row of cells persists to about 1200 microns from the apex (fig. 9), after which time the root-cap tissue is completely sloughed.

When living root tips are stained with IKI, the cells of the root cap give a positive test for starch (figs. 11 and 12). Starch grains are apparent in this tissue from the time the radicle emerges. With an increase in root length, the size and quantity of starch grains increases. When seedlings are allowed to grow until the appearance of the first leaves, there is a further increase in the amount of starch deposited in the root-cap cells. At no time during the growth of the primary root is there a noticeable decrease in the starch content of these cells as would be expected if it were being used up during growth. Experiments were designed to test this observation. Numerous seedlings were grown either on moist filter paper or in water culture and examined at frequent intervals. Both categories of roots were allowed to grow until the first leaves had begun to carry on photosynthesis. When the root caps of these roots were tested for the presence of starch, little difference in the amount of starch could be found.

A second experiment was designed to investigate the fate of the starch grains when the roots are severed from the food supply in the cotyledons. Seedlings with well-developed roots were chosen for the experiment. Twenty roots were excised just below the hypocotyl, measured, and placed on

numbered filter paper in a petri dish. Demineralized distilled water was added to the filter paper to keep the roots moist and living. As a control, 20 intact seedlings were measured and placed under the same growing conditions. After 20 hours in the dark at room temperature, 10 control roots and 10 excised roots were removed from the petri dish, measured and tested for the presence of starch. Root caps of all 10 control roots stained heavily with IKI indicating a large amount of starch still present in the root-cap cells. On the other hand, root caps of excised roots varied considerably in this respect. Four root caps gave a negative test for starch, two stained nearly as heavily as the controls, while the remaining four gave a positive test but contained fewer starch grains than the controls.

The remaining roots were placed back in the dark for another 24 hours. At the end of this time, the root caps were tested and there was a noticeable reduction in the amount of starch in all of the excised root tips. From numerous examinations it is evident that the root-cap cells of white mustard normally contain a large amount of starch which is not exhausted by the root in the early stages of growth as long as the cotyledons are intact. The results of these experiments support the view of Netolitzky (1935), that starch stored in the root cap is used only under conditions of extreme starvation.

2. Epidermis

Roots of white mustard have a uniseriate epidermis consisting of alternating rows of short and long cells. In transverse sections, the short cells are observed to be wedge shaped and in contact with two cortical cells (see fig. 3). The long cells situated between two short cells are observed to be radially elongated and to vacuolate much earlier than the short cells (figs. 3, 9 and 10). There are usually 2 to 3 long cell rows to every row of short cells. Differentiation of the epidermis into short and long cells occurs within a range of 24-60 microns from the root apex (Table I). Although these values are lower than those reported previously (Cormack, 1947, 1948), a different criterion was used. In this study, measurements were made from the top of the root-cap initials (see figs. 1, 2, and 4), and as soon as a difference in cell shape is obvious, the epidermal layer is considered differentiated. Previous values were obtained from epidermal strips including the root-cap tissue. When first differentiated, both cell types are densely protoplasmic and have a prominent nucleus (fig. 5). Many recent periclinal divisions adding cells to the root cap are obvious at this stage. As the root grows in length, the long cells undergo tangential enlargement whereas the short cells undergo anticlinal longitudinal divisions to keep pace with the increasing circumference of the root (fig. 6).

In the 20 roots examined, the long cells of the epidermis

begin to vacuolate at an average distance of 260 microns from the apex (see Table I and fig. 8), while the short cells remain densely protoplasmic until about 800 microns from the apex. At average distances of 827 and 1587 microns, one-half of the long and short cells respectively are completely vacuolated (Table I).

A characteristic feature of the short epidermal cells is their ability to "push out" small papillae which become the root hairs. At the time the papillae are formed, most of the long epidermal cells are completely vacuolated. Living roots were examined with a Spencer student microscope (10X ocular) and the distance from the root apex to the first-formed papillae determined with a calibrated eyepiece micrometer. The range of values obtained (1770-2655 µ's), is slightly higher than when permanent slides are examined (1327-2383 µ's). Likewise, the mean of the measurements using living roots is higher than the corresponding mean for permanent sections (see Table I). During fixation and embedding, many of the epidermal cell walls become buckled making it difficult to tell by the examination of prepared sections, where the first papillae arise. Therefore, the values recorded for fresh roots are considered more reliable. The short epidermal cells are considered to be entering their maturation phase when the root-hair papillae begin to appear. Since the long cells normally do not produce root hairs, they are considered mature when they are completely vacuolated.

PLATE III.

Figure 10. (X1300) Transverse section at 500 μ's from the root apex showing the contrast in vacuolation of short and long cells of the epidermis. The triangular intercellular space below the short cell borders on the space system of the cortex. (s) short cell (l) long cell (r) root-cap cells

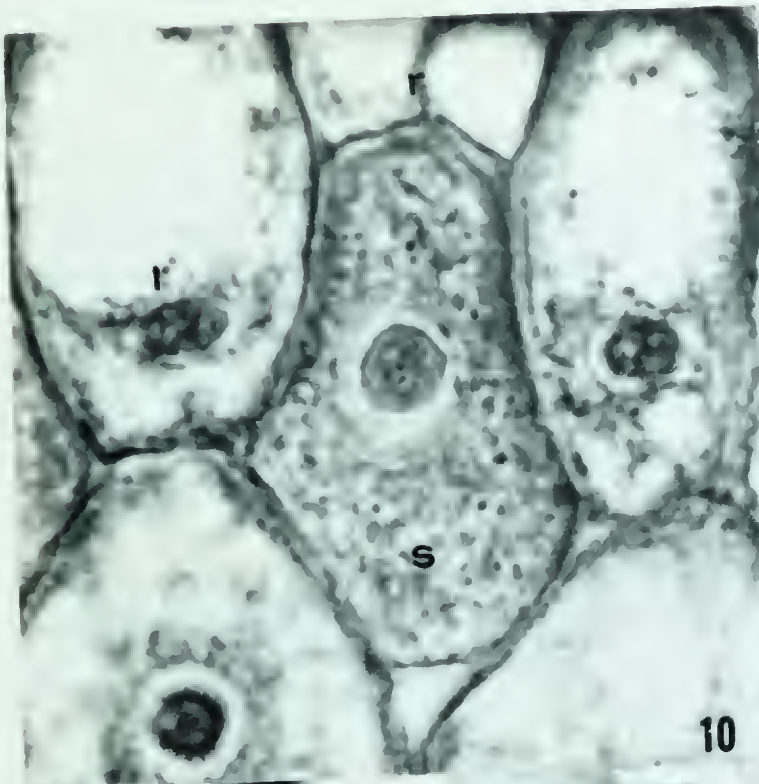
Figure 11. (X400) Median longitudinal section of the root tip stained with Crystal Violet. Large starch grains are evident in the root-cap cells.

Figure 12. (X1300) Fresh section of root-cap tissue stained with IKI showing starch grains which are stained a deep purple.

Figure 13. (X1300) Permanent transverse section of the root tip approximately 40 μ's from the apex. At this level, the first intercellular spaces appear in the middle cortical tissue.

Figure 14. (X1300) Intercellular spaces of the same root at 120 μ's from the apex.

Figure 15. (X1300) Intercellular spaces at 600 μ's from the apex. At the top of the photomicrograph, the triangular spaces bordering on the epidermal cells are apparent.



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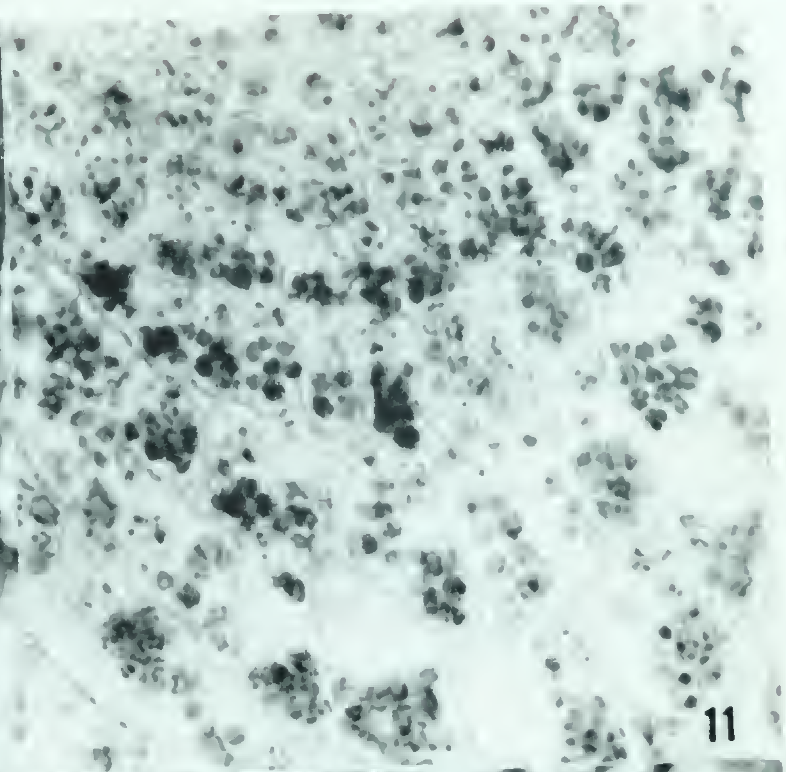

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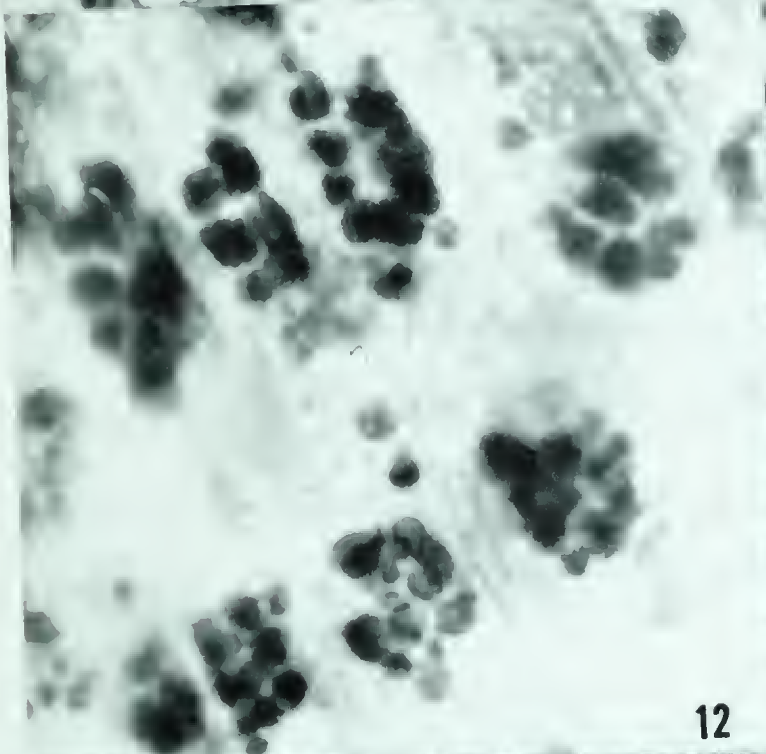

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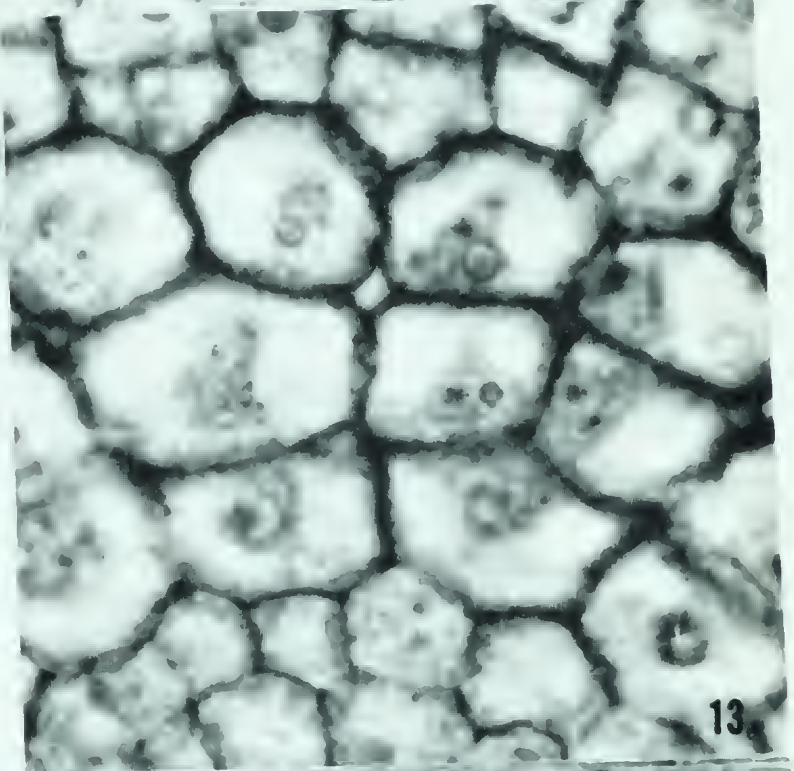

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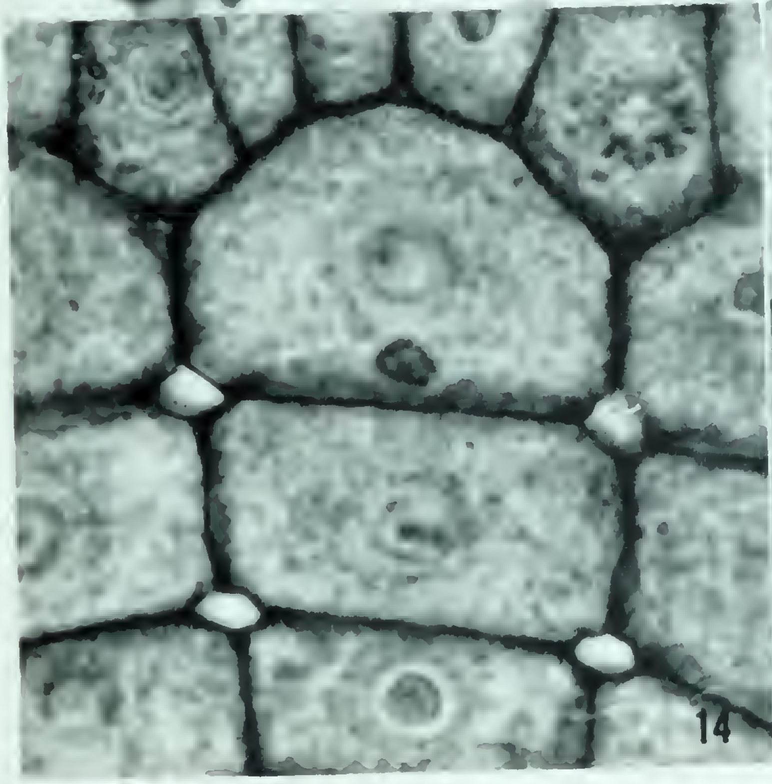

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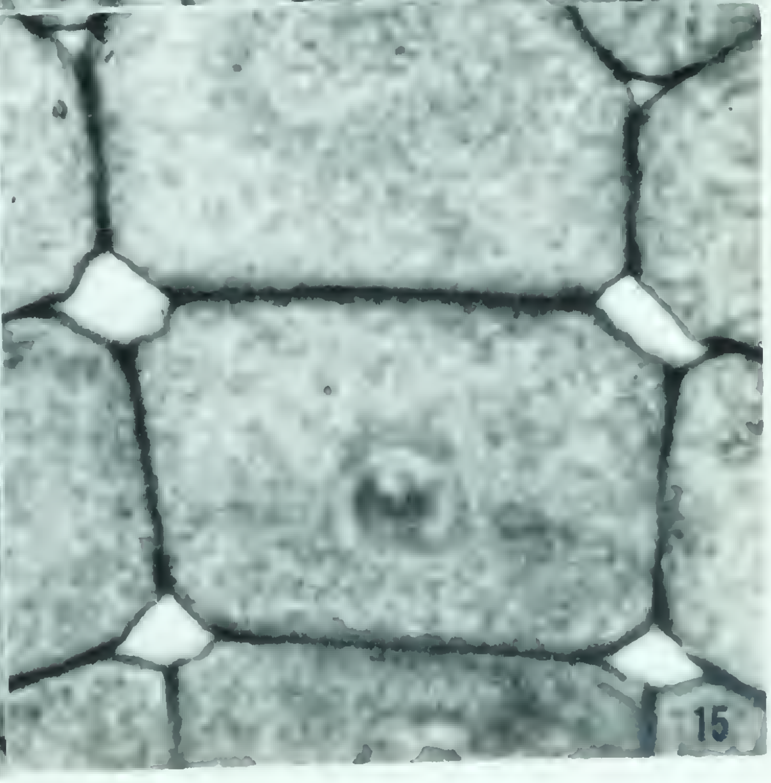

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TABLE I

Levels of tissue differentiation and appearance of other features in the epidermis and root cap of roots grown under normal conditions. All measurements are in microns and refer to distances from the root apex. Range and mean values were obtained from a minimum of 15 roots and a maximum of 20 roots.

	Range	Mean
Long and short ep. cells diff.	24 - 60	48
Long cells begin vac.	139 - 307	260.6
Short cells begin vac.	487 - 1363	800.6
One-half long cells compl. vac.	643 - 1087	827.8
One-half short cells compl. vac.	1327 - 1723	1587.8
Root-hair papillae appear (perm)	1327 - 2383	1923.8
Root-hair papillae appear (fresh)	1770 - 2655	2197.8
Rootcap - from apex to tip	163.4 - 228	185.2
Rootcap - 4+ cell layers appear in transverse sections	204 - 336	283.2
Rootcap - 3 cell layers	288 - 492	397.6
Rootcap - 2 cell layers	420 - 804	617.6
Rootcap - 1 cell layer	876 - 1404	1137.6

Abbreviations:

compl. - complete
ep. - epidermal
diff. - differentiate
perm. - permanent slide
vac. - vacuolated

From this study, it is obvious that both epidermal cell types mature approximately 2000 microns beyond the point at which they are first differentiated.

3. Cortex

In white mustard roots, the five rows of cortical cells, inclusive of the endodermis, are typically opposite in arrangement (Cormack, 1947), (see fig. 3). The row of cells destined to become the endodermis and regarded as being "cambium-like" by Williams (1947), gives rise by successive cell divisions to the remaining four rows. When first formed, the cortical cells appear brick-shaped in transverse section and are densely protoplasmic (figs. 5 and 6). By frequent anticlinal divisions in the outer rows of cells, the cortex keeps pace with the increase in circumference of the root. With growth and maturity, the outer cells become dome-shaped in transverse view (figs. 7 and 8), and eventually highly vacuolated.

As the cortical cells continue to grow, they round-up and pull apart from each other at the corners, producing small intercellular spaces which enlarge with the growth of the root (see figs. 13, 14 and 15). From the beginning of their development, these spaces measure an average of 3-4 μ's across their widest diameter. Measurements of intercellular spaces between the fourth and fifth (outermost) row of cortical cells show an increase in average size with increasing distance from the root apex (Table VII). At 840 μ's from the root apex, the average size of the spaces has increased

to approximately 10 µ's. At distances beyond this, accurate measurements of intercellular space size could not be made, because the highly vacuolated plant tissue becomes distorted with fixing and cutting.

Although the conspicuous vacuolation of the cortical parenchyma can be judged only subjectively (Popham, 1955), a general centripetal trend is obvious (Table II). An exception, the earlier vacuolation of the second row (from the epidermis) as compared to the outer row, might possibly be explained on the basis of cell position. The outer row is in closer contact with the external environment and therefore within access of a readier oxygen supply. Vacuolation of the remaining cell rows is definitely centripetal and can be explained on the basis of decreasing age of cells and increasing proximity to the food conducting elements with each layer of cells inward.

Staining living roots with IKI confirmed the fact that like most dicotyledonous roots, the cortical cells of white mustard are parenchymatous storage cells containing an abundance of small starch grains.

The innermost row of cells is referred to as the endodermis (fig. 3). In its embryonal stage, the cells of this layer resemble the majority of meristematic cells in that they are protoplasmic and capable of dividing. Since the endodermis occupies an interfacial position between two extremely different tissue systems _i.e._ the vascular cylinder

and the cortex, many functions have been attributed to it. As the primary and subsequent phases develop, the endodermis becomes a barrier to the rapid diffusion of water and solutes across the root tissues (Priestley and North, 1922; Van Fleet, 1961). The ability of the endodermis to prevent the leakage of water from the vascular cylinder back to the surface of the root may account in part for the ascent of water in plants (Priestley and Tupper-Carey, 1922; Van Fleet, 1961). The characteristic endogenous origin of branches on roots and the exogenous origin of branches on stems, may in part be explained by the presence or absence of an endodermis (Priestley, 1922).

In white mustard roots, a continuous cylinder of endodermal cells becomes apparent approximately 40 μ's from the root apex (Table II), but the Casparian strip representing the primary phase of maturation of the endodermis (Priestley and North, 1922), is not laid down until approximately 2000-3000 μ's from the apex. It has been demonstrated by solubility tests and staining reactions that the Casparian strip is most probably composed of lignin and fatty acids (Priestley and North, 1922; Van Fleet, 1961). In roots with a small diameter, The Casparian strips are very narrow and difficult to identify in prepared sections because of the buckling of the thin cell walls during fixation and cutting (Esau, 1940; Heimsch, 1951). Because of this difficulty, fresh sections were cut transversely and stained with

TABLE II

Levels of tissue differentiation and appearance of other features in the cortex of roots grown under normal conditions. All measurements are in microns and refer to distances from the root apex. Range and mean values were obtained from a minimum of 15 roots and a maximum of 20 roots.

	Range	Mean
Endodermis - compl. cyl.	24 - 48	38.4
Endodermis - Casp. strip (f.h.)	2000 - 3000	-
First intercellular space	24 - 48	39.2
Cortical par. - high. vac. (outer or 5th row)	655 - 1110	801.4
Cortical par. - high. vac. (4th row)	595 - 1063	788.6
Cortical par. - high. vac. (3rd row)	631 - 1231	914.2
Cortical par. - high. vac. (2nd row)	665 - 1291	986.2
Cortical par. - high. vac. (inner or endodermis)	919 - 1795	1315.0

Abbreviations:

compl. - complete
cyl. - cylinder
high. - highly
vac. - vacuolated
f.h. - free-hand
par. - parenchyma

Polychrome Methylene Blue (Van Fleet, 1950), to obtain information on the level of the first appearance of the Casparian strip. The Casparian strips indicated in fig. 22 are stained in this manner.

4. Vascular cylinder

In the primary root, the vascular cylinder or stele constitutes all the tissue inside the endodermis (see fig. 3). The outermost row of cells of the vascular cylinder, the pericycle, is recognizable as a continuous cylinder approximately 60 µ's from the root apex (Table III). When all primary tissues are mature, the pericycle gives rise by repeated cell divisions to the development of secondary roots.

In the present study, only primary vascular tissue (tissue derived from the initial cells in the meristem) is considered. The terms protoxylem and protophloem are applied to the xylem and phloem cells which mature in advance of the other vascular elements and which by their position mark the pattern of subsequent maturation of primary vascular tissues. The location of these first vascular cells may be referred to as the protoxylem and protophloem poles.

Contrary to general opinion, the metaxylem differentiates slightly before the protoxylem and well in advance of the protophloem (Table III). Figure 16 shows the first differentiated metaxylem cell which appears elliptical and enlarged in transverse section. Figure 17, approximately 60 µ's from the apex, shows further differentiation and enlargement of

PLATE IV.

Figure 16. (X1300) Permanent transverse section of the root tip approximately 50 µ's from the root apex. The limits of the vascular cylinder are visible and the first-differentiated metaxylem cell as indicated by the arrow has enlarged.

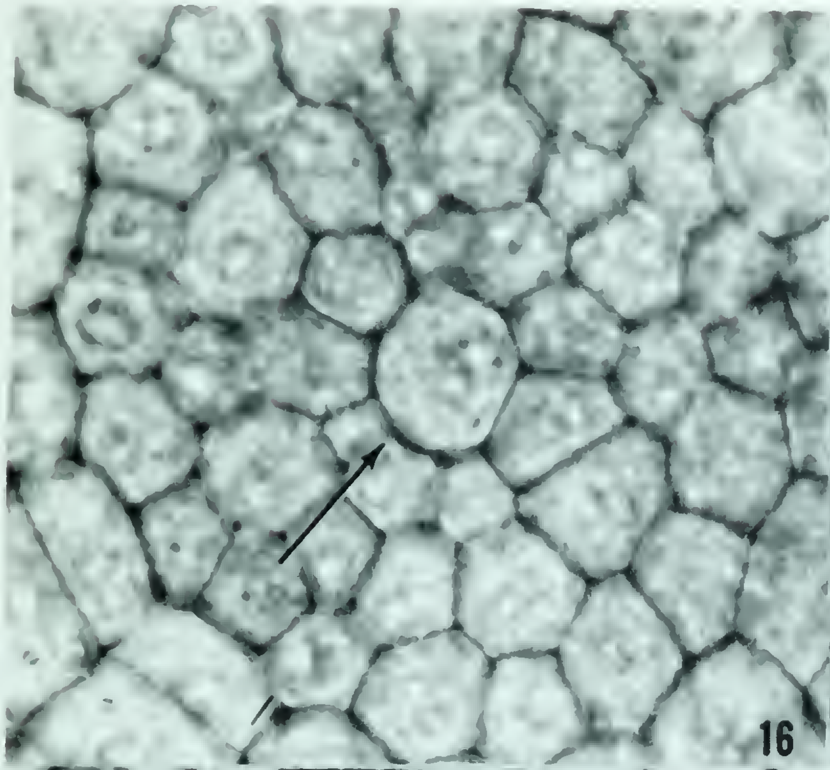
Figure 17. (X600) The same root approximately 80 µ's from the apex showing the diarch xylem plate confined by the two protoxylem poles as indicated by the arrows.

Figure 18. (X1200) Transverse section of the root tip approximately 3000 µ's from the apex. At this level, one protoxylem element (as indicated by the arrow) is mature.

Figure 19. (X600) Transverse section of the same root shown in Figure 18 at approximately 4000 µ's from the apex. Arrows indicate a mature protoxylem element at each protoxylem pole.

Figure 20. (X400) Fresh section of white mustard root tip approximately 2 cm. from the apex, showing the centripetal maturation of the primary xylem.

Figure 21. (X1600) Permanent median longitudinal section of the root tip showing typical secondary wall thickenings of the protoxylem.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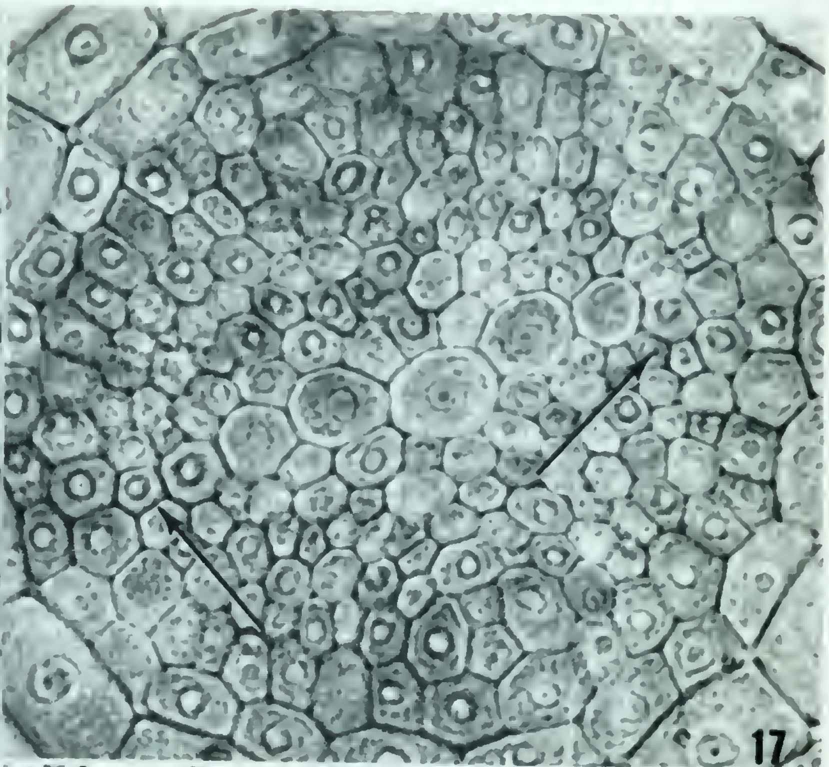
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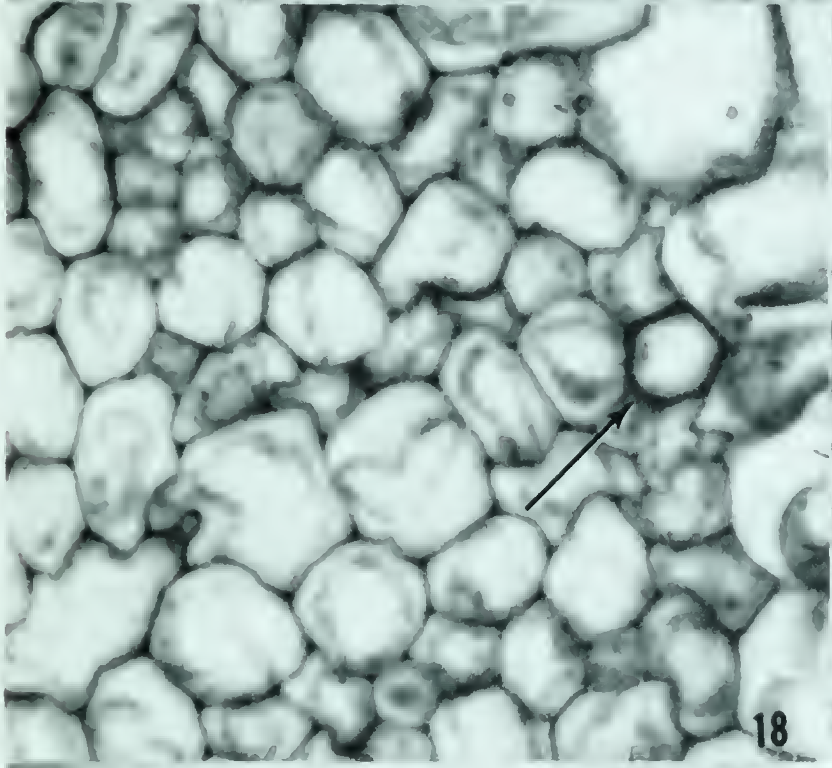
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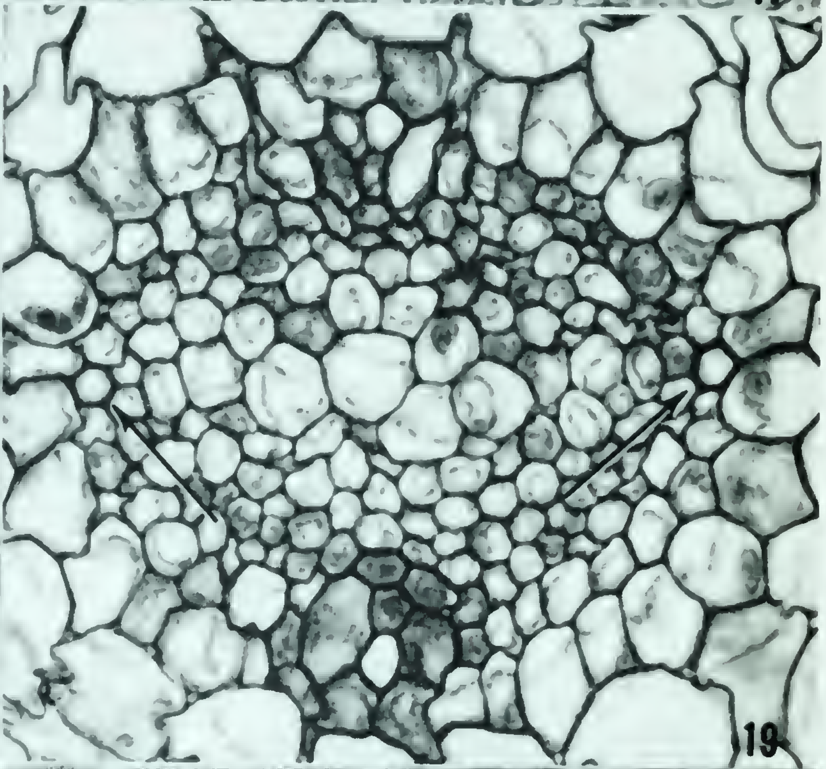
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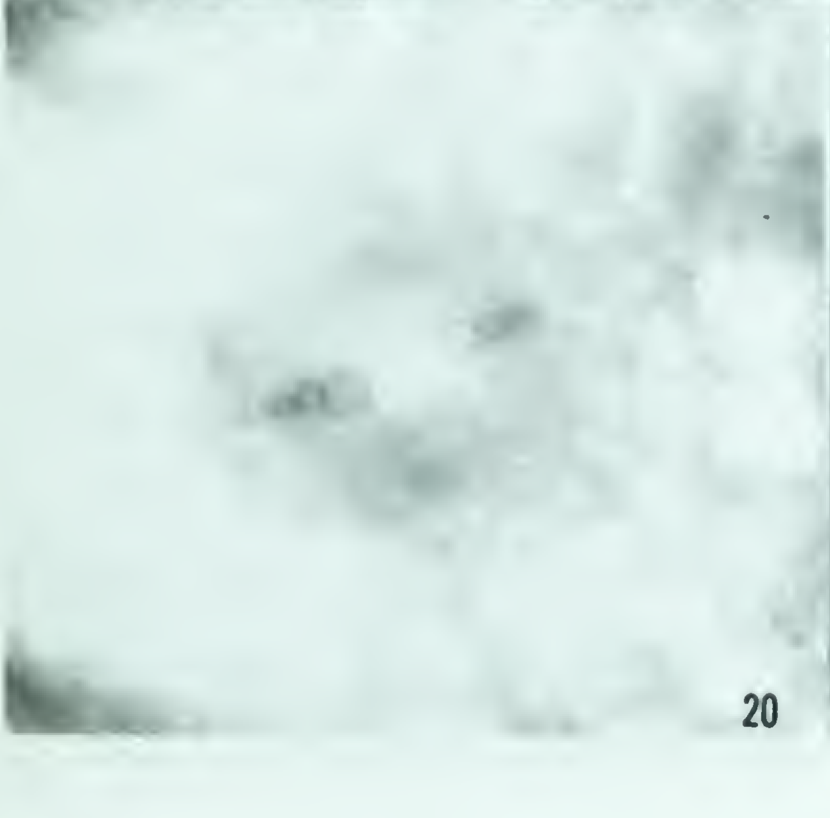
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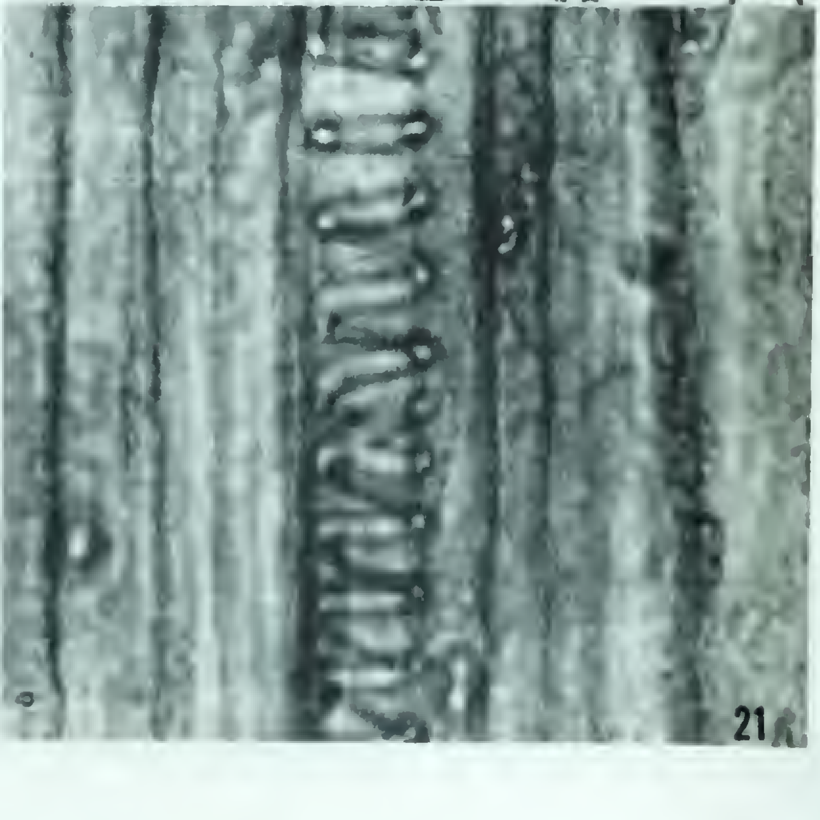
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cells on either side of the first elliptical metaxylem cell. At this point, both protoxylem cells (as indicated by the arrows) are differentiated and mark the outer limits of the diarch xylem plate. Cells on either side of the xylem plate remain undifferentiated and meristematic at this time.

While the xylem cells continue to enlarge and vacuolate, changes in cells at right angles to the xylem establish the location of the protophloem poles. Differentiated protophloem sieve tubes are easily recognizable from the surrounding cells by their pentagonal shape and heavier stained appearance as viewed in transverse section (Esau, 1941; Heimsch, 1951). At an average distance of 278 µ's from the root apex, a sieve tube cell at one pole is differentiated (Table III). Often to establish the level of first differentiation as accurately as possible, a well-developed sieve tube was followed through successively younger sections until the initial signs of differentiation were noticed. In fig. 23 the arrow at the right points to a sieve tube which is differentiated already and the arrow at the left points to a sieve tube which is just beginning to differentiate. This establishes the alternate arrangement of primary vascular tissue characteristic of roots. Maturation of the first-differentiated sieve tubes proceeds rapidly, one generally maturing in advance of the other (see figs. 24 and 25). Sieve tubes are regarded as mature when they show a scarcity of stainable contents and the absence of a nucleus (Esau, 1941; Heimsch, 1951). In

PLATE V.

Figure 22. (X2000) Transverse section of a living root tip at approximately 2-3 mm. from the apex stained with Polychrome Methylene Blue. Arrows indicate the Casparian strip which is stained a deep blue.

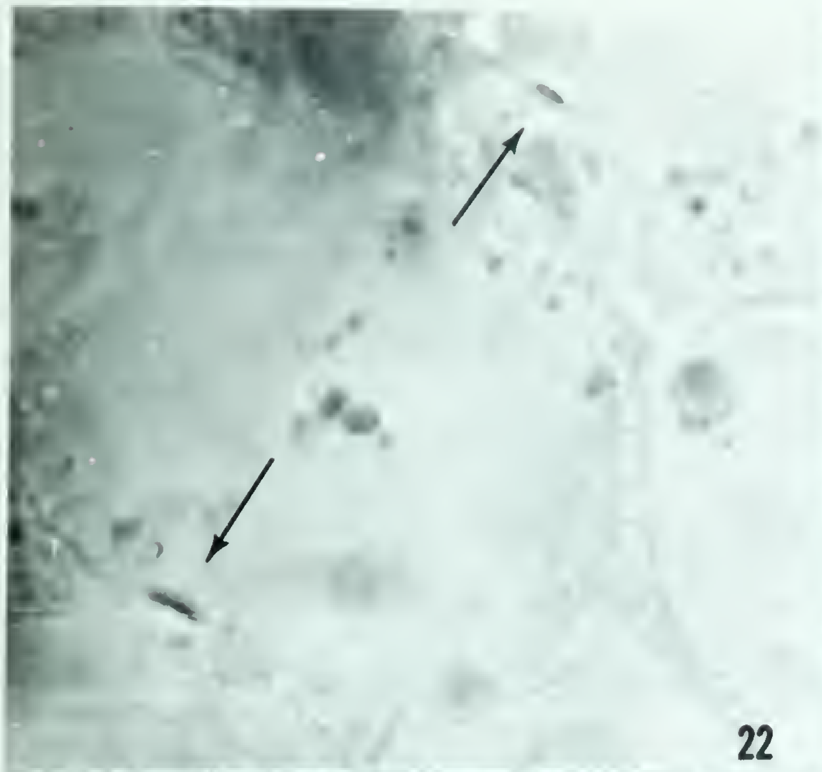
Figure 23. (X600) Permanent transverse section at 300 µ's from the apex. At the right, a sieve tube cell is easily recognizable while at the left another has just differentiated.

Figure 24. (X600) The same root approximately 50 µ's beyond the level shown in Figure 23. The first-differentiated sieve tube to the right shows conspicuous vacuolation while the other to the left is now easier to see because of its heavier staining walls.

Figure 25. (X600) Transverse section of another root at a similar level as that in Figure 24. The heavier staining walls of the sieve tubes are more striking in this root.

Figure 26. (X600) Section at 500 µ's from the apex showing a mature sieve tube at both protophloem poles.

Figure 27. (X600) Transverse section approximately 4000 µ's from the apex. Arrows indicate sieve tubes that have differentiated centripetally in relation to the first two.



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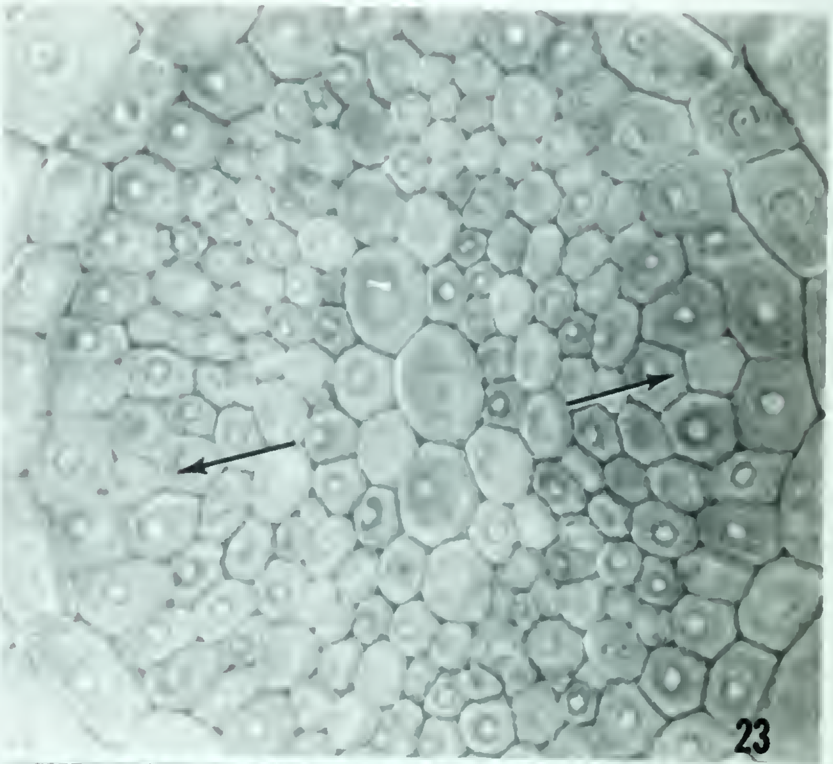

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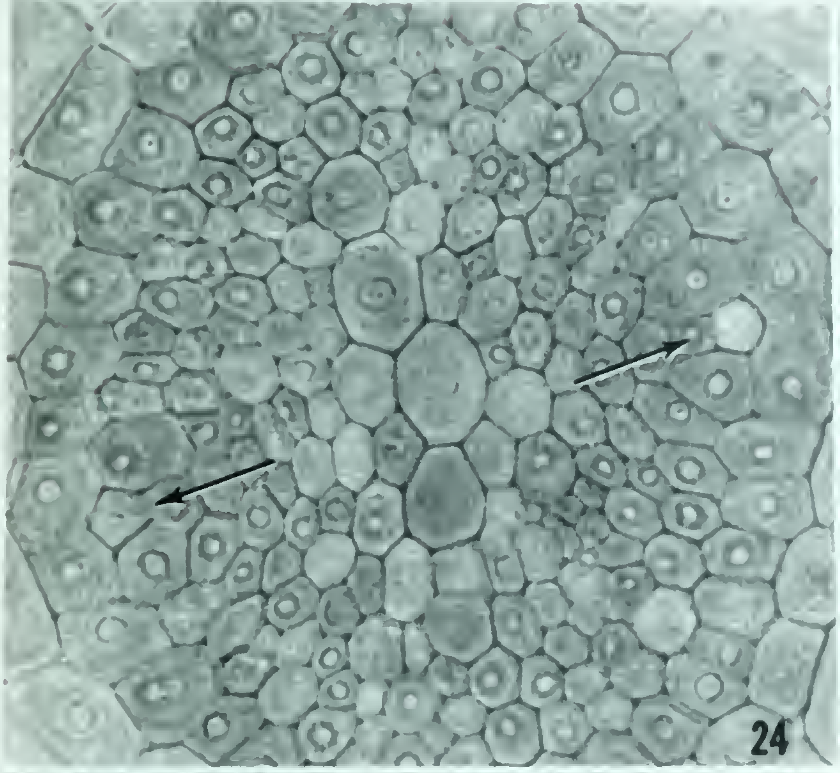

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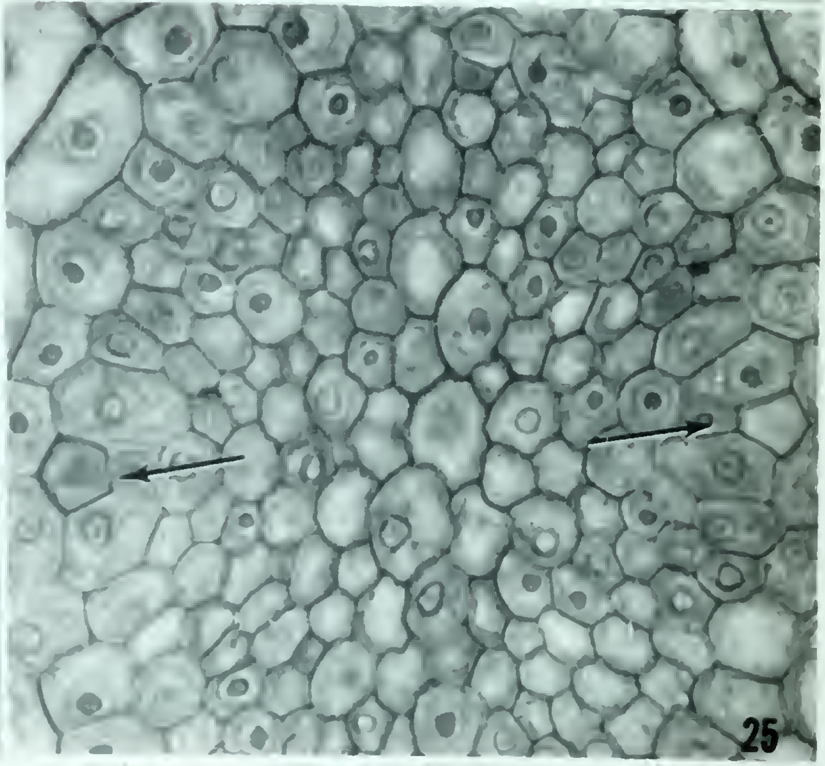

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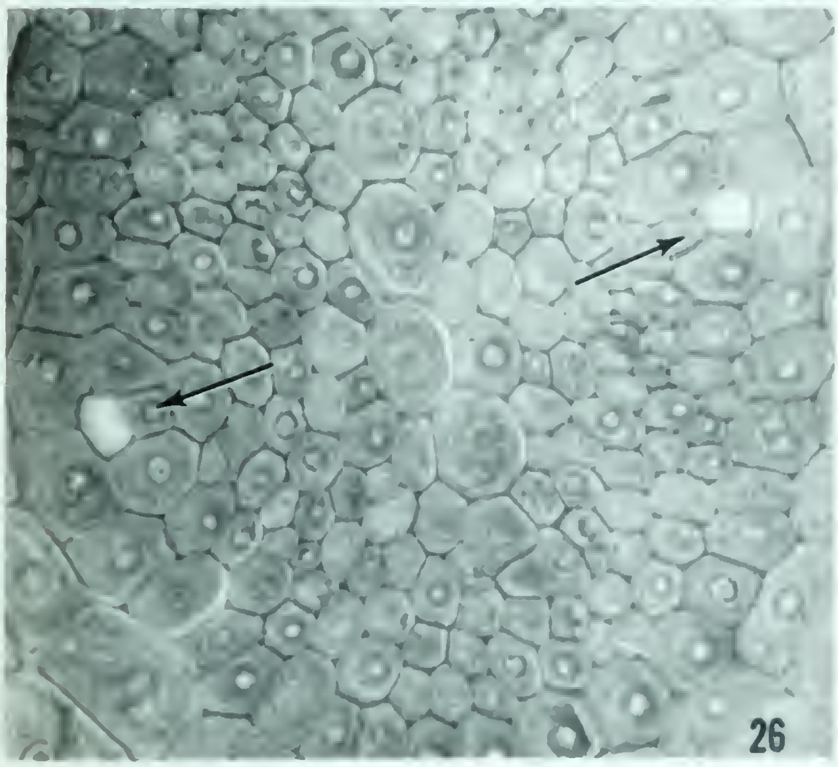

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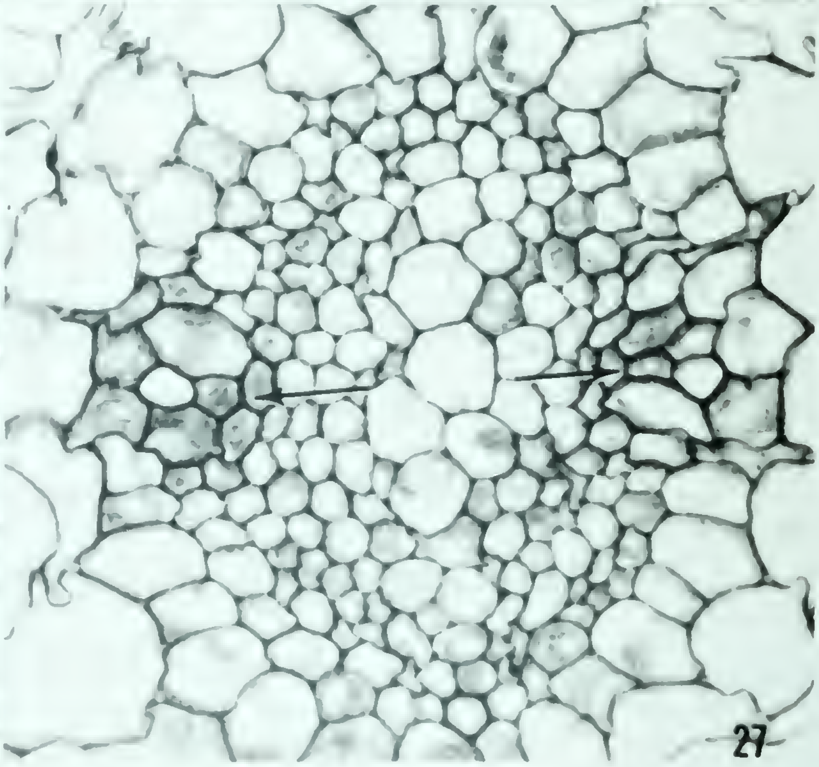

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the roots examined in detail, the first sieve tube to mature is found at an average distance of 400 μ's from the apex, while the second is found just slightly beyond this level (Table III). Figure 26 shows the root at a level where two sieve tubes are mature. Additional sieve tubes are observed to differentiate centripetally with respect to the first sieve tube cells at approximately 3000 μ's from the apex (see fig. 27).

After the sieve tubes at the protophloem poles have differentiated and matured, further changes indicative of maturation occur in the cells of the xylem plate. Lignification of the cell walls is held to be an early indication of maturity in xylem cells. However, they are not considered fully mature until they have lignified secondary walls, and have lost both nucleus and cytoplasm (Esau, 1940; Heimsch, 1951). Because of the difficulty encountered in obtaining suitable transverse sections of the living root, a new method was tried. A longitudinal slit through the epidermis and cortex was made with a pair of fine needles under a dissecting microscope. The tissue was laid back exposing the intact vascular cylinder. A saturated solution of Phloroglucinol in 95% ethanol was added followed by a drop of concentrated HCl (Johansen, 1940). The treated sections were then examined with a Spencer student microscope and the distance from the root-cap initials to the first signs of lignification measured with a calibrated eyepiece micrometer (Table III Lignified xylem f.h.). The

first deposits of lignin in the cell walls appear light red when stained with Phloroglucinol. The degree of lignification is indicated by the intensity of the staining reaction. Lignin in well-developed xylem vessels stains a deep cherry red with this stain. Further measurements using a saturated solution of Benzidine in glacial acetic acid as the stain, gave approximately the same results (Table III). With this stain, lignified cell walls stained a yellow-orange, the intensity depending upon the amount of lignin deposited. The lignified secondary wall thickenings of the protoxylem cells are always helical, annular, or intermediate in type (fig. 21). These maturing cells are first recognized at approximately 2800 µ's from the root apex and are located at the periphery of the vascular cylinder. Invariably, lignified wall thickenings are observed at one protoxylem pole slightly in advance of the other. Since at this stage they still contain cytoplasm and a nucleus, they are not considered fully mature. The protxylem element indicated in fig. 18 is the first mature xylem element in this root. In the roots examined, the occurrence of the first mature protoxylem element ranges from 2052-4274 µ's from the root apex (Table III). Once again, due to the difficulty in obtaining suitable transverse sections of this root, it was impossible to make accurate measurements of further maturing xylem elements. However, by examination of a few good slides some indication is obtained as to how further maturation takes

TABLE III

Levels of tissue differentiation and maturation in the vascular cylinder of roots grown under normal conditions. All measurements are in microns and refer to distances from the root apex. Range and mean values were obtained from a minimum of 15 roots and a maximum of 20 roots.

	Range	Mean
One sieve tube diff.	204 - 336	278.4
Two sieve tubes diff.	252 - 348	308.8
Mature sieve tube	336 - 468	401.6
Two mature sieve tubes	360 - 504	432.8
Metaxylem diff.	36 - 60	47.2
Protoxylem diff.	36 - 72	56
Xylem - lig. wall (f.h.)	1600.8 - 4287.2	2793.9
Xylem - lig. wall (f.h.) - Benz.	1814.4 - 4422.6	3237.8
Mature protoxylem	2052 - 4274	3157.8
Pericycle - compl. cyl.	36 - 72	56.8

Abbreviations:

cyl. - cylinder
compl. - complete
diff. - differentiate
f.h. - free-hand
Benz. - Benzidine
lig. - lignified

place. As indicated in fig. 19, the xylem element at the opposite protoxylem pole is next to mature. From fig. 20, a fresh transverse section, the centripetal maturation of further xylem elements can be followed. The larger metaxylem cells toward the centre of the section lignify and mature after the smaller protoxylem elements. In the present study, roots were not grown for a sufficient length of time to observe the complete lignification and maturation of the entire xylem plate, but it is obvious from fig. 20 that although the central metaxylem cells are the first to differentiate (fig. 16), they are the last to mature.

5. Summary of levels of primary tissue differentiation and maturation in white mustard root tips

When the data recorded in Tables I, II, and III are summarized by diagramatic representations of the root tip, the difference between the levels of differentiation and maturation of the various tissues is obvious. Figure 28 shows the spatial relationship of tissue differentiation and maturation in longitudinal view, while figs. 29 (a), (b) and (c) show the same relationship in transverse section. Figures 28 and 29 (a) clearly show that the majority of primary tissues differentiate within 100 µ's of the root apex. At this stage the root cap consists of four rows of cells surrounding the rest of the root apex (fig. 29a); the epidermis is differentiated into short and long cells; the endodermis or "proendodermis" (Popham, 1955), is recognizable as a

distinct cylinder completing the five rows of cells typical of the cortex; and intercellular spaces appear between cells of the inner cortex. Within the vascular cylinder, the pericycle is well marked off as a single row of cells and the position of the diarch xylem plate limited on either side by two differentiated protoxylem cells and characterized in the centre by the enlarging and vacuolating metaxylem cells is fairly well defined.

Immediately above this level (approximately 280 μ's from the root apex), (see fig. 28), one sieve tube at one protophloem pole is differentiated already while a second sieve tube is beginning to differentiate at the other protophloem pole directly opposite. Figure 29 (b) shows the condition of the growing point at approximately the 400 u level. Here an immature sieve tube is indicated at one protophloem pole and a mature one at the opposite pole. The earlier differentiation and maturation of a sieve tube at one pole in advance of the other is invariably the case in white mustard root tips. Further changes at this level in the root include a slight diminishing in thickness of the root-cap tissue and the conspicuous vacuolation of the long epidermal cells (fig. 28). In contrast to the early vacuolation of the long epidermal cells, vacuolation of the short epidermal cells begins approximately 900 μ's from the root apex (fig. 28).

Subsequent developmental changes at a still higher level,

FIGURE 28.

Diagram of longitudinal section of a root tip of white mustard illustrating certain features of differentiation and maturation. Levels indicated for each tissue are the averages of measurements made on 15-20 roots. (diagram after Esau, 1941)

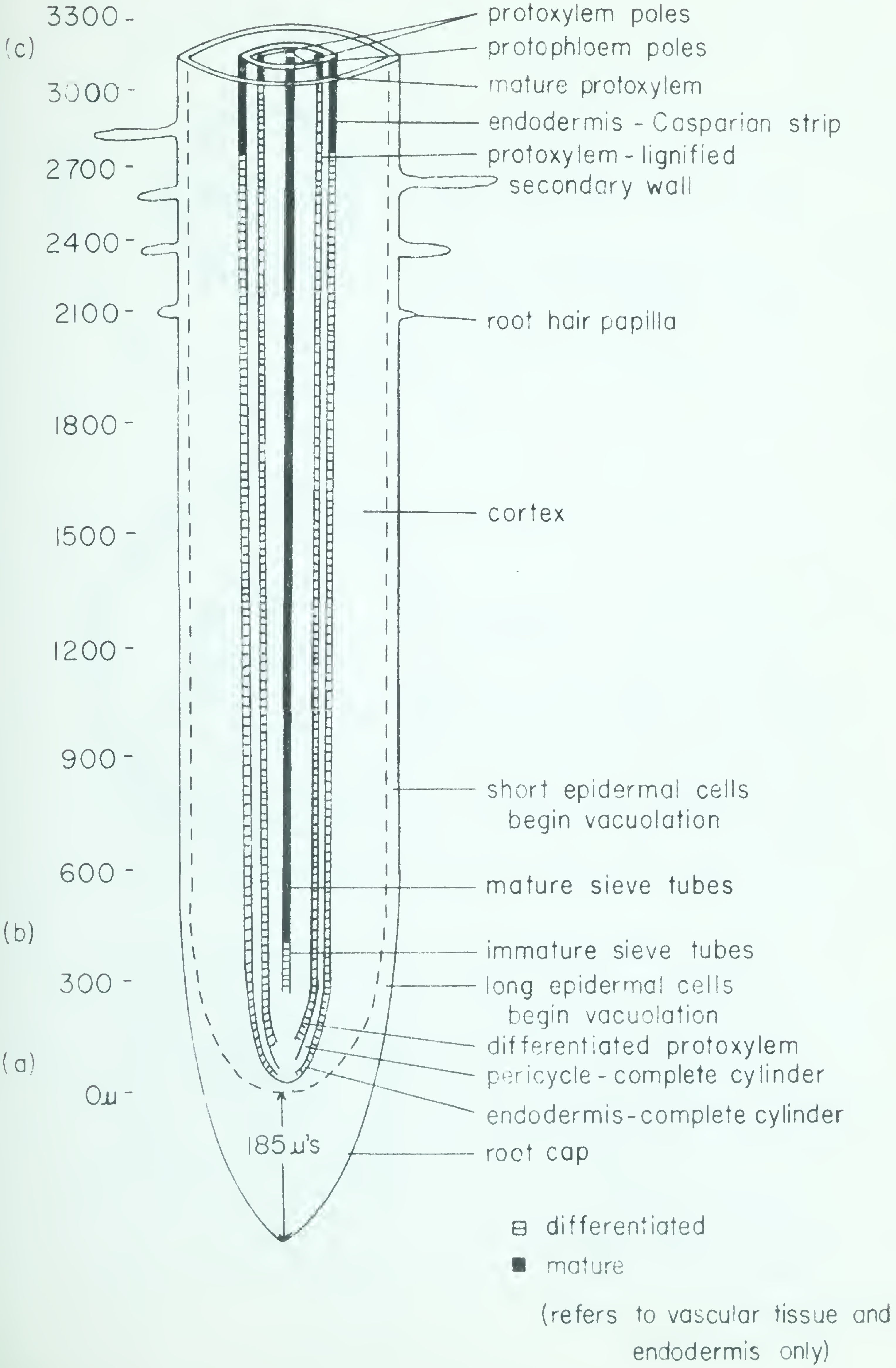
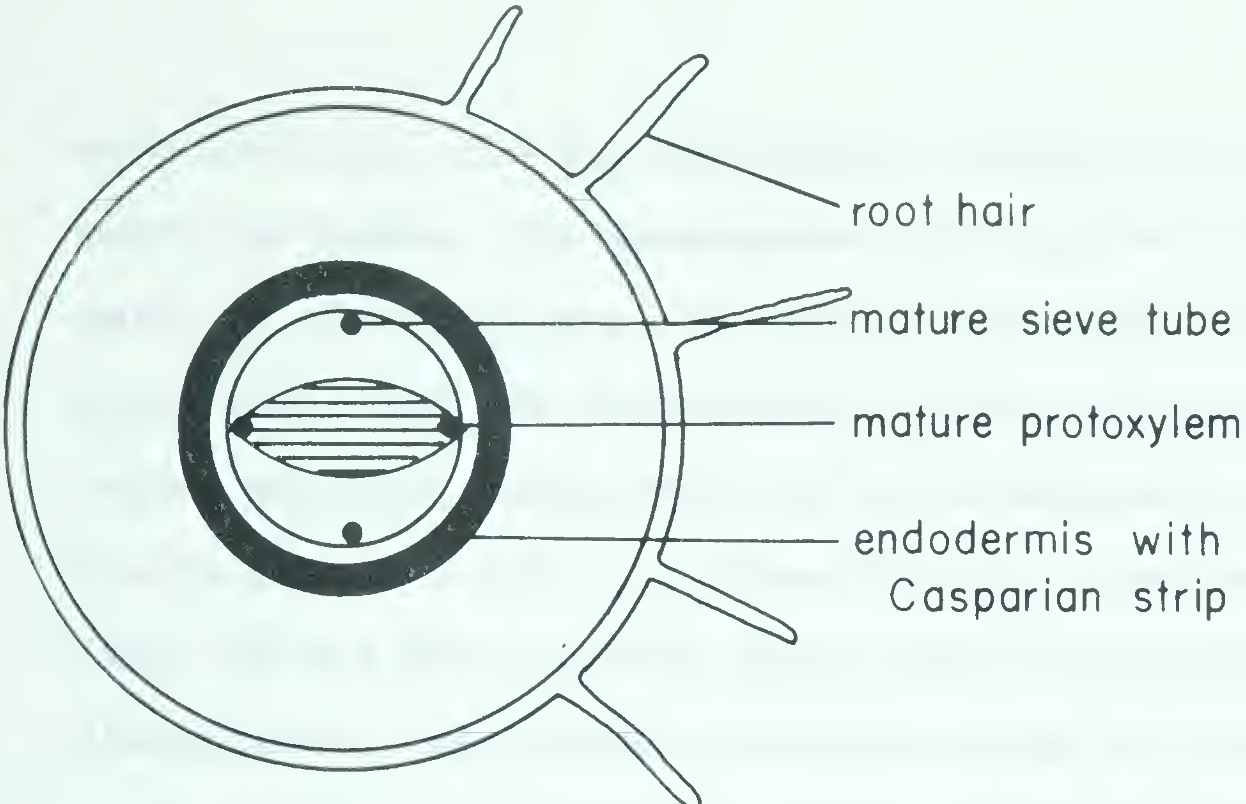
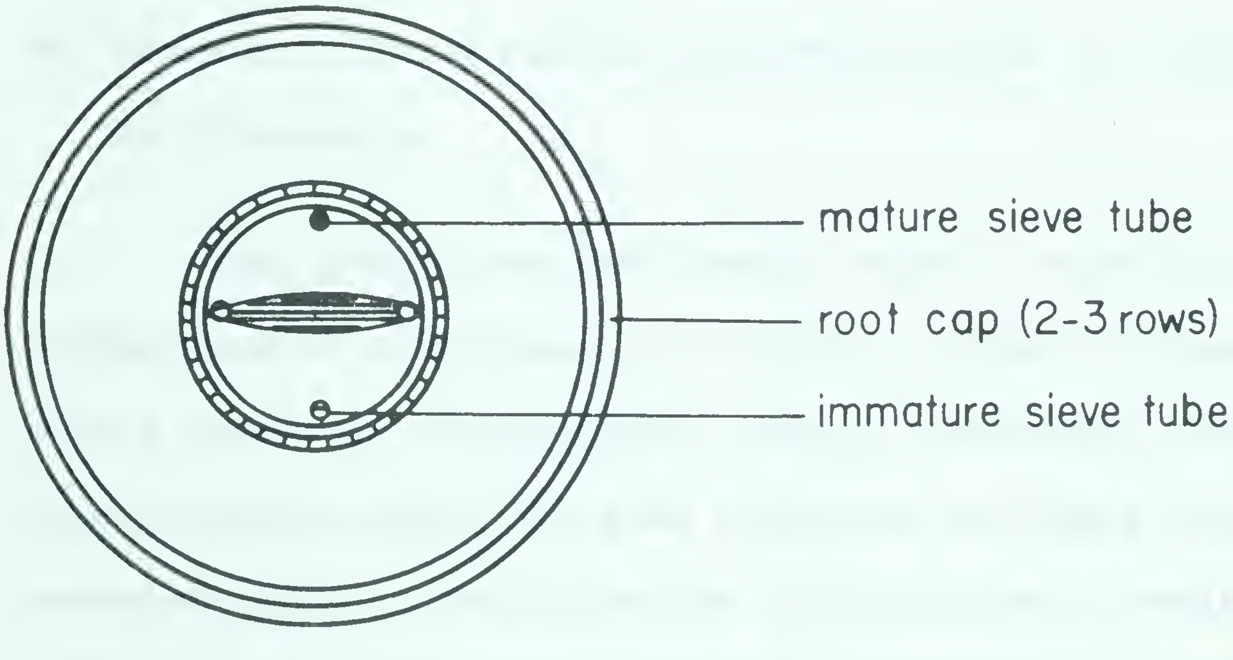

3300 -
(c)
3000 -
2700 -
2400 -
2100 -
1800 -
1500 -
1200 -
900 -
600 -
(b)
300 -
(a)
0µ -
185 µ's
protoxylem poles
protophloem poles
mature protoxylem
endodermis - Casparian strip
protoxylem - lignified secondary wall
root hair papilla
cortex
short epidermal cells begin vacuolation
mature sieve tubes
immature sieve tubes
long epidermal cells begin vacuolation
differentiated protoxylem
pericycle - complete cylinder
endodermis - complete cylinder
root cap
⊟ differentiated
■ mature
(refers to vascular tissue and endodermis only)

FIGURE 29.

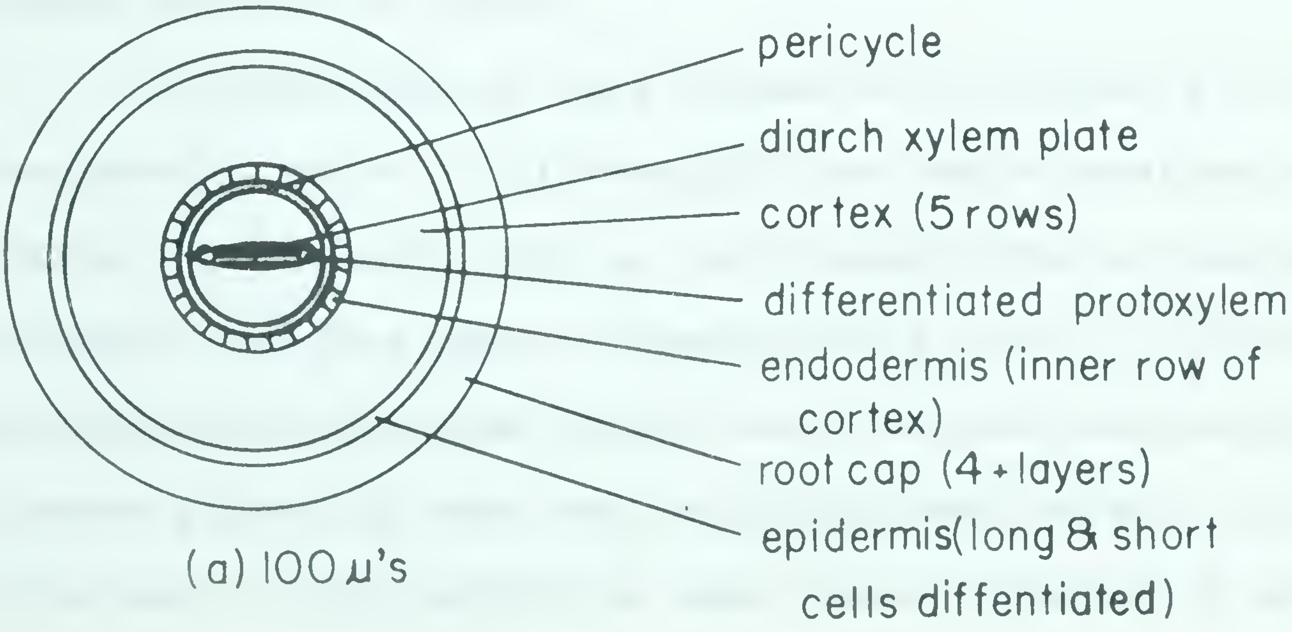
Diagrams of transverse sections of the same root shown in Figure 28 illustrating certain features of differentiation and maturation at three different levels, (a), (b), and (c) indicated in Figure 28.



(c) 3200 µ's



(b) 400 µ's



(a) 100 µ's

some 2100 μ's from the root apex, involve the absence of root-cap tissue, the development of papillae by the short cells of the epidermis, the early lignification of the protoxylem cells and the deposition of fatty substances on the radial and transverse walls of the endodermal cells to form the Casparian strip. At about 3000 μ's from the root apex (fig. 28 and 29c), beyond which level measurements were discontinued, all primary tissues except the metaxylem are mature.

B. Tissue Differentiation and Maturation in Experimental Environments

The growth rate of roots under a specific set of experimental conditions is rather constant (Appendix I). Roots grown in non-aerated culture solutions invariably have the slowest growth rate as compared to those in either the aerated culture solutions or the moist-air environment. Roots grown in the latter environment consistently show the fastest growth rate in 24 hours.

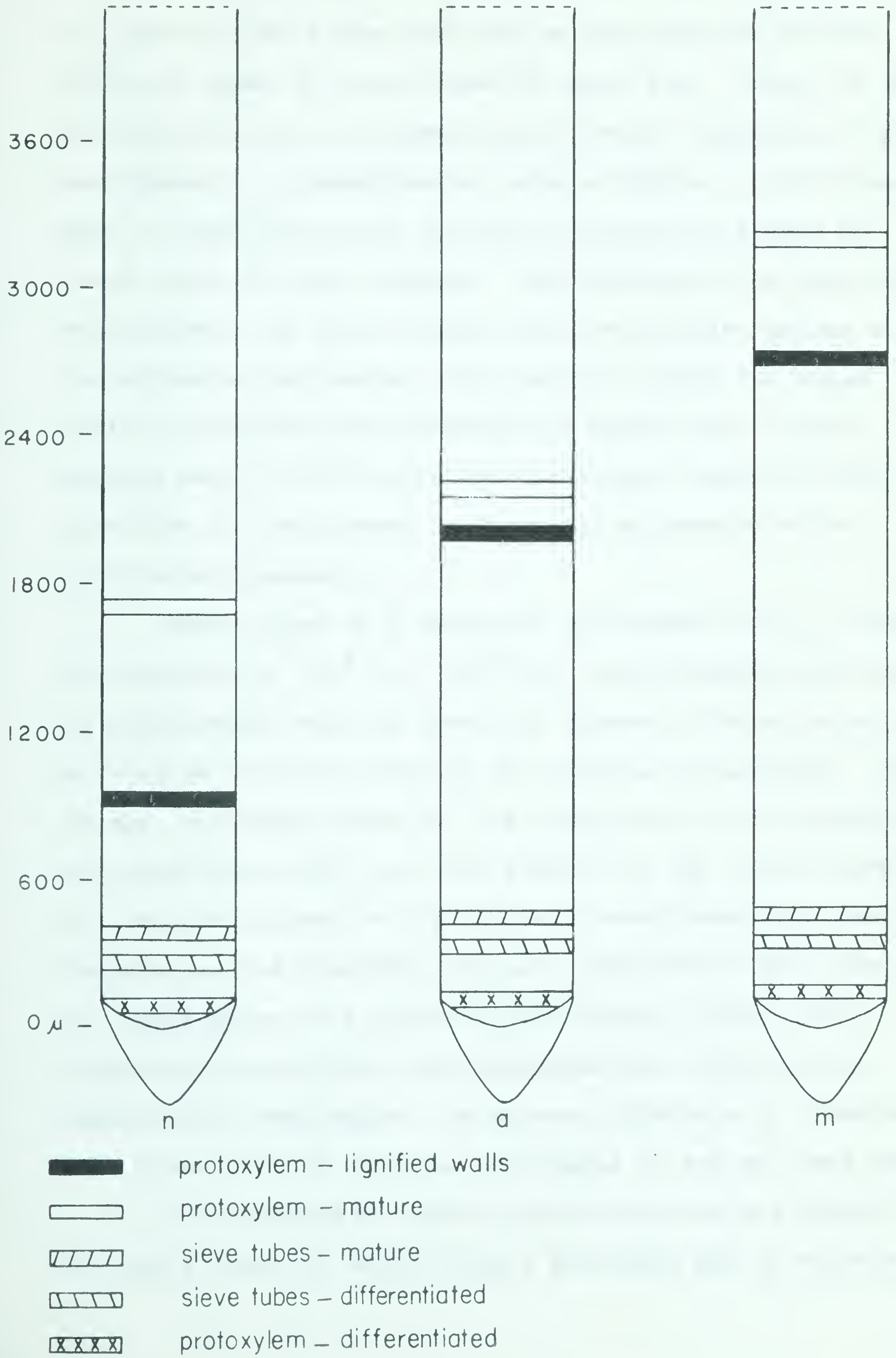
A comparison of data presented in Tables I-VI indicates the general trend of differentiation and maturation of tissues closest to the root apex in the slowest-growing roots and furthest from the apex in fast-growing roots. Although the distance from the apex to the level of differentiation of tissues generally does vary with the environment, this variation is only slight in most cases. Figure 30 shows

diagramatically the levels of differentiation and maturation of primary vascular tissues grown in non-aerated water culture (n), aerated water culture (a), and in the normal moist-air environment (m). From the diagrams, it is apparent that differentiation of protoxylem varies only slightly from root to root. Other tissues not indicated in fig. 30 which show this same slight range of variation with environment include the differentiation of the metaxylem and short and long cells of the epidermis, the presence of the pericycle and endodermis as complete cylinders of cells, and the appearnce of the first intercellular spaces between cells of the inner cortex. Figure 30 also shows the consistency of differentiation and maturation of sieve tubes of the protophloem in all the roots examined.

In a similar manner, fig. 30 shows the different levels at which lignification and maturation of protoxylem cells takes place. Both processes occur closer to the root apex in roots grown in the non-aerated culture solutions and furthest from the apex in roots grown in a moist-air environment. Although not represented in fig. 30, the first appearance of the root-hair papillae is also influenced greatly by the environment. Roots grown in non-aerated solutions fail to develop root hairs. Root-hair papillae first appear approximately 1000 μ's closer to the apex in aerated culture solutions as compared to roots grown in a moist-air environment. The best development of root hairs

FIGURE 30.

Diagrams showing the levels of differentiation and maturation of some of the primary vascular tissues in roots grown in three different environments, namely; (n) non-aerated water culture solutions, (a) aerated water culture, and (m) moist-air environment. All levels are the averages of measurements presented in table form.


3600
3000
2400
1800
1200
600
0 µ
n
a
m
protoxylem – lignified walls
protoxylem – mature
sieve tubes – mature
sieve tubes – differentiated
protoxylem – differentiated

as judged by the number and area of root covered is consistently shown by roots grown in moist air. Figure 30 shows that the root cap is present in all roots regardless of the environment. A comparison of data in Tables I and IV does show a slight reduction in amount of root-cap tissue of roots grown in water culture. The environment is also shown to influence the time at which cell vacuolation begins in the epidermis and cortex. The level at which the cells begin to conspicuously vacuolate in roots grown in non-aerated water culture solutions is always closer to the root apex than in roots grown in moist-air or aerated water culture environments.

Roots grown in a moist-air environment over a range of temperatures (37° F. - 100° F.), were examined and detailed measurements made of levels of tissue differentiation and maturation of roots grown at the extreme temperatures. The levels, including those for the lignification and maturation of protoxylem cells, are very similar to the values recorded for the roots grown in the moist-air environment at room temperature and therefore are not presented in table form. All roots grown in a moist-air environment within this temperature range have well-developed root caps and an abundance of root hairs. No marked difference in intercellular space size or shape is noticeable in any of these roots.

The sequence of tissue differentiation and maturation for roots grown in water culture solutions and in moist air

TABLE IV

Levels of tissue differentiation and appearance of other features in the epidermis and root cap of white mustard roots grown in water culture. All measurements are in microns and refer to the distance from the apex. Range and mean values based on 10-15 roots.

	Non-aerated Water Culture		Aerated Water Culture	
	Range	Mean	Range	Mean
Long and short ep. cells diff.	24 - 36	27.6	24 - 36	28.8
Long cells begin vac.	103 - 523	278.2	271 - 691	411.8
Short cells begin vac.	247 - 979	532.6	643 - 1159	898.2
One-half long cells compl. vac.	295 - 835	575.8	607 - 1159	844.6
One-half short cells compl. vac.	679 - 1219	846.2	763 - 2011	1124.6
Root-hair papillae appear (fresh)	none		590 - 1534	1177.1
Rootcap - from apex to tip	129.6 - 194.4	157.9	153.9 - 194.4	173.6
Rootcap - 4+ cell layers appear in transverse sections	156 - 276	212.4	168 - 372	241.2
Rootcap - 3 cell layers	240 - 396	301.2	240 - 528	322.8
Rootcap - 2 cell layers	300 - 624	447.6	384 - 708	499.2
Rootcap - 1 cell layer	576 - 912	758.4	516 - 1102	821.8

TABLE V

Levels of tissue differentiation and appearance of other features in the cortex of white mustard roots grown in water culture. All measurements are in microns and refer to the distance from the root apex. Range and mean values based on 10-15 roots.

	Non-aerated Water Culture		Aerated Water Culture	
	Range	Mean	Range	Mean
Endodermis - compl. cyl.	24 - 36	28.8	36 - 48	46.8
First intercellular space	12 - 36	18.4	24 - 48	36
Cortical par. - high. vac. (outer or 5th row)	151 - 751	529.3	307 - 763	519.8
Cortical par. - high. vac. (4th row)	115 - 739	501.1	415 - 907	563.8
Cortical par. - high. vac. (3rd row)	127 - 811	499.8	427 - 1075	627.6
Cortical par. - high. vac. (2nd row)	187 - 727	536.6	475 - 1219	703.5
Cortical par. - high. vac. (inner or endodermis)	319 - 991	603.8	463 - 1411	807.0

Abbreviations:

compl. - complete
cyl. - cylinder
high. - highly
vac. - vacuolated
par. - parenchyma

TABLE VI

Levels of tissue differentiation and maturation in the vascular cylinder of white mustard roots grown in water culture. Measurements are in microns and refer to distance from the root apex. Range and mean values were obtained from a minimum of 10 and a maximum of 15 roots.

	Non-aerated Water Culture		Aerated Water Culture	
	Range	Mean	Range	Mean
One sieve tube diff.	72 - 360	162.0	132 - 300	212.4
Two sieve tubes diff.	108 - 384	199.2	144 - 336	252.0
Mature sieve tube	252 - 504	363.6	324 - 468	362.4
Two mature sieve tubes	264 - 564	387.6	336 - 492	409.2
Metaxylem diff.	12 - 24	18.0	24 - 48	39.6
Protoxylem diff.	24 - 48	37.2	24 - 72	46.8
Xylem - lig. wall (f.h.)	615.6 - 1587.6	977.4	1377 - 3169	2021.8
Mature protoxylem	1332 - 2376	1782.0	1620 - 2940	2221.3
Pericycle - compl. cyl.	36 - 60	45.6	48 - 84	69.6

Abbreviations:

cyl. - cylinder
compl. - complete
diff. - differentiate
f.h. - free-hand
lig. - lignified

within the temperature range indicated is remarkably similar to that shown in figs. 28 and 29 (a), (b), and (c).

C. Intercellular Spaces and their Content

1. Space size

Three permanent slides of serial transverse sections of control roots grown at atmospheric pressure and roots grown under reduced pressure were randomly selected for intercellular space measurements. At intervals of 120 μ's beginning at the root apex and continuing basally for a distance of 840 μ's, the widest diameter of each intercellular space between the fourth and fifth (outermost) row of cortical cells was determined using an oil immersion lens (100X ocular) and a calibrated eyepiece micrometer. The range and mean values are recorded in Table VII. The difference in intercellular space size at comparable levels in the two categories of roots is not striking. To a distance of 480 μ's from the root apex, the difference in average size is never greater than 1.0 μ. From 480-840 μ's from the root apex, differences in mean values range from 1.0-2.5 μ's. Examination of all slides prepared for this study reveals no noticeable difference in shape of the intercellular spaces of roots grown under reduced pressure as compared to the controls.

2. Intercellular space content

For all experiments and observations to be described, sections were cut free hand with a sharp razor blade from living root tips.

TABLE VII

A comparison of intercellular space size in roots grown under atmospheric and reduced pressure. Range and mean values (in microns) based on 60 individual measurements.

Distance From Apex	Atmosperic Pressure		Reduced Pressure	
	Range	Mean	Range	Mean
120	1.7 - 8.6	5.4	2.4 - 9.6	5.5
240	1.8 - 10.8	5.9	2.4 - 14.4	6.6
360	1.8 - 14.4	6.7	3.0 - 13.4	7.3
480	2.2 - 13.4	7.0	3.0 - 14.4	8.6
600	2.4 - 12.2	7.4	3.0 - 18.2	9.9
720	2.4 - 20.6	8.1	3.0 - 20.6	10.1
840	3.6 - 18.0	9.8	3.6 - 20.6	10.8

When thin cross sections of living stem and root tips are viewed in water under the microscope, the small intercellular spaces frequently appear black. If commented on at all, this discoloration has been attributed to air trapped in the spaces. However, evidence is accumulating that suggests that at an early stage of development, intercellular spaces contain something more than, or, besides air. Most recently, Sorokin (1958), considers the intercellular spaces in young pea stems to contain a solid and refers to it as the "intercellular substance" or "intercellular tubular material".

Because of the controversy associated with the identification of this substance (Sorokin, personal communication), preliminary experiments were designed to determine whether the intercellular spaces of white mustard root tips contain something besides a gas, and if so to try and establish its chemical and physical nature.

i) Distribution

Transverse and longitudinal sections were cut and examined under ordinary light conditions with a Zeiss photomicroscope. The photomicrographs (figs. 32, 33 and 34) compare almost exactly with those obtained by Sorokin (1958) in her study of the intercellular spaces of pea stems. Within approximately 1500 μ's of the root apex, nearly every cortical intercellular space as well as nearly every small triangular space bordering on the short epidermal cells is

PLATE VI.

(All photomicrographs are of living white mustard root tips which were cut free hand with a sharp razor blade.)

Figure 32. (X2500) Transverse section approximately 1.0 mm. from the apex. All intercellular spaces appear dark.

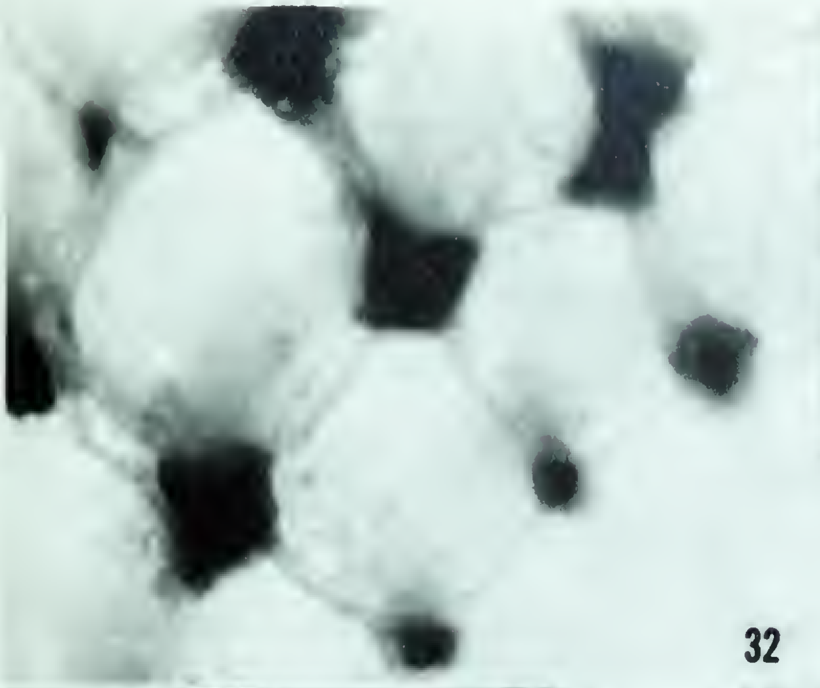
Figure 33. (X2500) Section at a slightly higher level showing some empty intercellular spaces in the middle cortical tissue.

Figure 34. (X2800) Cortical tissue just before the region of root hairs showing some empty intercellular spaces.

Figure 35. (X2000) Section at approximately the same level as that shown in Figure 34 showing the dark-appearing material "hanging on" in the spaces towards the epidermis.

Figure 36. (X2500) A section at the same level as in Figure 35 showing remnants of the dark material in the inner cortical spaces.

Figure 37. (X1800) Transverse section in the region of root hairs showing all the intercellular spaces of the cortex devoid of the dark-appearing material.



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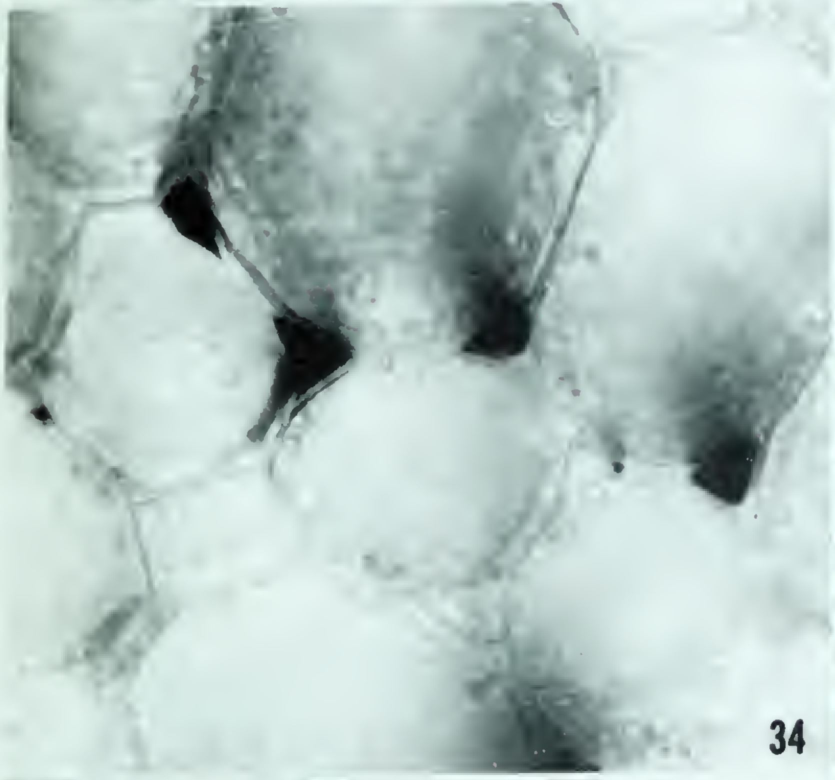

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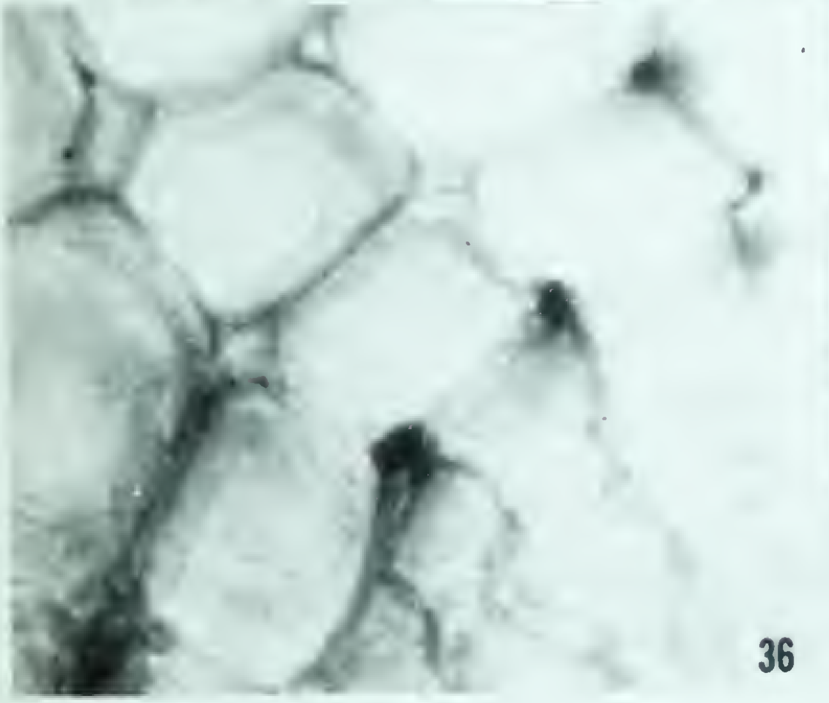

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filled with this material (see fig. 32). This is the case whether transverse sections are mounted in distilled water, glycerin, or a variety of other media. Further from the root apex, the material disappears from the inner cortical spaces (figs 33 and 34), but is persistent in the spaces bordering the vascular cylinder (fig. 36), and particularily in the small triangular spaces bordering the epidermis (fig. 35). However, by the time the region of root-hair development is reached, it has disappeared from all the intercellular spaces except a few in the outer cortex (fig. 37). This characteristic distribution pattern is found in all roots examined.

ii) Preliminary experiments to determine the physical nature of the material

The next series of experiments are designed to produce changes in the intercellular material by lowering the atmospheric pressure. They were undertaken on the assumption that if the blacking is due to air trapped in the intercellular spaces then a visible change should occur in these small cavities after subjecting the root section to reduced pressure. The general procedure is as follows: following microscopic examination of the intercellular spaces, thin free-hand transverse sections are mounted either on moist filter paper or on a dry microscope slide and placed inside a vacuum dessicator which is attached through a manometer to a vacuum pump. The pressure is reduced to 5-10 mm. of mercury for 10-15 minutes

and then suddenly released. Figures 38 and 39 show the appearance of a typical cross section before and after treatment. Although slightly shrivelled, the intercellular material appears much the same after treatment (fig. 39), as it did before (fig. 38). In the case of transverse sections placed on dry microscope slides, the cortical cells appear dessicated but on addition of a few drops of water they regain some of their turgidity allowing photographs to be taken (see fig. 39). Only those intercellular spaces which appear to contain something in them prior to treatment show any signs of contents upon re-examination. Likewise, all the intercellular spaces that are empty before treatment (fig. 38), are still empty after treatment (fig. 39). These observations are typical of the results consistently obtained, and strongly suggest that the intercellular spaces contain some other substance besides a gas.

The next series of experiments are designed to test the effects of temperature on the intercellular material. The simplest method is to allow the sections to dry on the microscope stage following initial examination of the intercellular spaces. The cells become dessicated and the section contracts. Small shrivelled remnants of the material can be seen before and after water is added to the section. Sections dried on a warming plate for periods ranging from one-half to one hour show the same results (fig. 40).

Transverse sections placed in distilled water on a

PLATE VII.

(All photomicrographs are of living white mustard root tips which were cut free hand with a sharp razor blade.)

Figure 38. (X2000) Transverse section of white mustard root tip before being placed in vacuum dessicator.

Figure 39. (X2000) Same section as shown in figure 38 after being placed in vacuum dessicator. Water was added in order to photograph the section. The intercellular material is still present.

Figure 40. (X2200) Appearance of the intercellular material and cells of the cortex after being heated on a warming plate. Water was again added to prepare the section for photography. The material was obvious before and after adding the water.

Figure 41. (X2500) Appearance of intercellular material in sections that had been brought to boiling point three times on a microscope slide. Only a slight swelling was noticed in the material.

Figure 42. (X2400) Section of the root tip showing the complexity of the surface of the material. Note particularily the space in the upper left corner.

Figure 43. (X1800) The complex hexagonal structure with scalloped edges was found only in this particular section.


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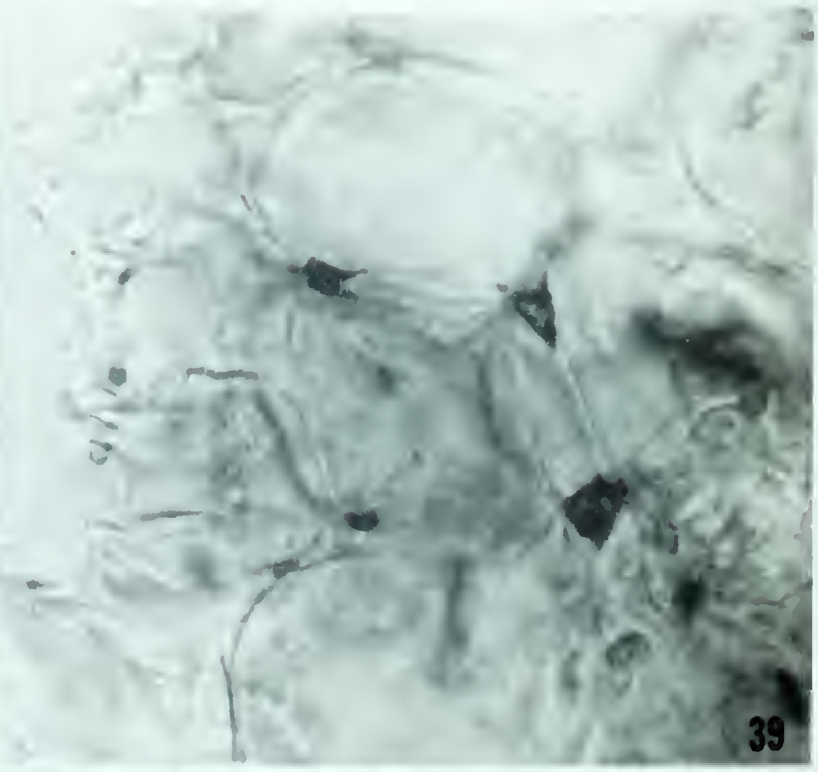
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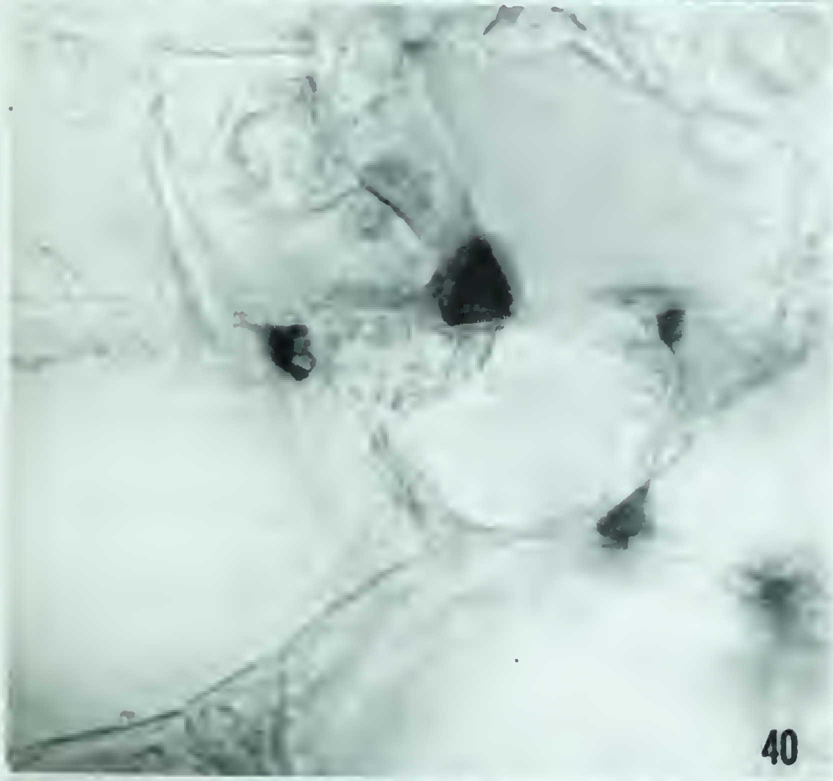
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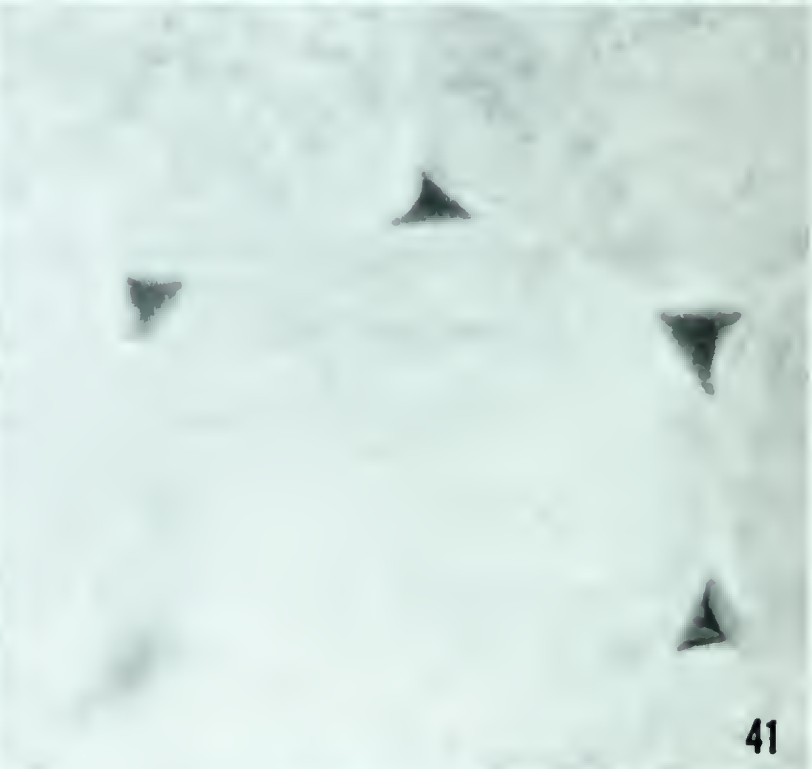
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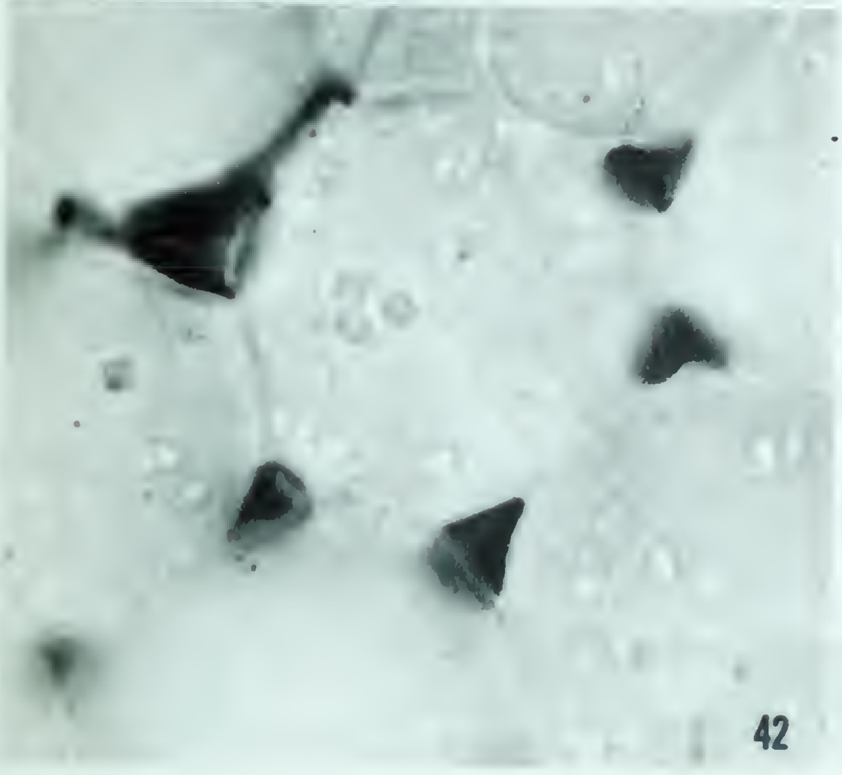
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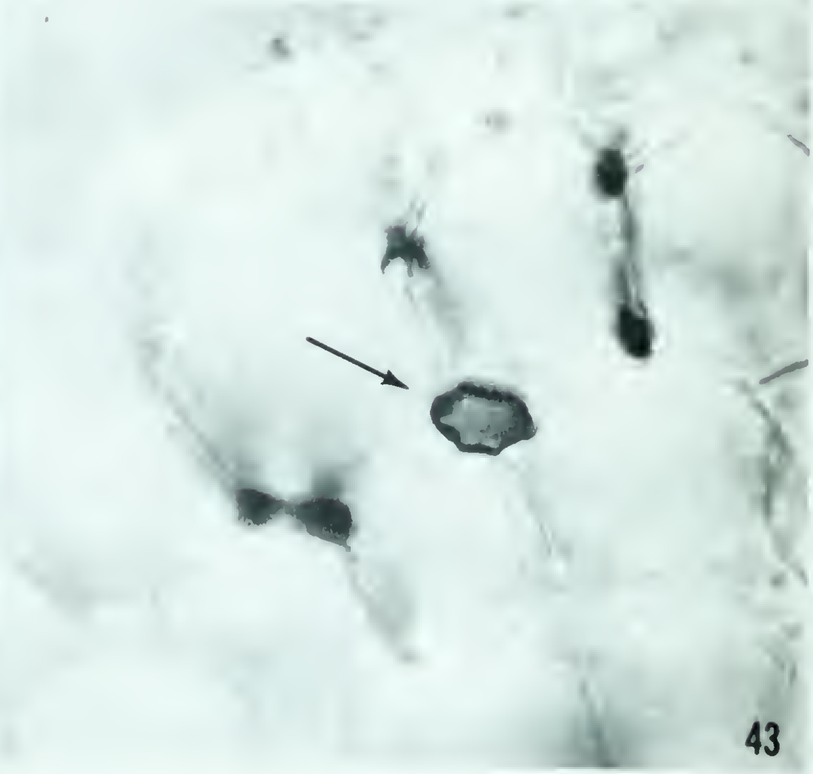
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microscope slide and brought to boiling point three or more times before examining under the microscope, show only a slight swelling of the material upon immediate examination. By the time the photographs are taken, little or no swelling is apparent. Figure 41 shows the partially destroyed cells and the appearance of the intercellular material after this treatment.

Further evidence that the intercellular spaces contain something more than a gas is obtained by microscopic examination of a number of transverse sections. One section (fig. 43), shows a complex hexagonal structure with a scalloped outer layer and a lighter interior. Whether the shape of this structure is due to the way the section is cut or whether it reflects the complexity of the intercellular material is impossible to determine. Further evidence of a complex surface unlike that of a gas-water interphase is shown by figure 42.

iii) Solubility and other properties of intercellular material

The effects of various chemical reagents were tested by perfusing fresh sections gradually with the reagent directly under the microscope and drawing it off by filter paper. The contents of the spaces is readily soluble in absolute and 95% ethanol, chloroform, acetone, xylol, ethyl ether, absolute methanol, and absolute tertiary butyl alcohol. The most interesting results are given by 95% ethanol in that some of

the tissue sections show an evolution of small bubbles from the intercellular contents. Sorokin (1958), also made this same observation. Shortly after this, the contents of the spaces dissolve completely.

Transverse sections mounted directly into a number of acids also give some interesting results. Glacial acetic acid causes a fast dissolution of all intercellular space contents. Sulphuric acid (67-72%) causes a change in viscosity of the intercellular material. It becomes semi-fluid, assumes peculiar shiny structures, some of which are shown in fig. 44, and then finally rounds-up into spherical bubbles which are not soluble in 95% ethanol or xylol. These results are identical to those recorded by Sorokin (1958).

The reaction of the intercellular space contents to a number of alkalis is nearly identical in all cases. In some sections the inner part of the material becomes dissolved leaving the outer part intact, when 4% NaOH, 3.5% NH_4OH, or 10 M. KOH is added. In other sections, the material is not affected.

iv) Staining reactions of intercellular space contents

The presence of lipids is demonstrated using a number of lipid-specific stains. Reactions and references for the stains are given in Table VIII. A saturated solution of Sudan IV dissolved in propylene glycol imparts a slight red color to much of the intercellular material. Swelling of

TABLE VIII

Reaction of intercellular material to various stains and histological tests.

Substrate	Reagent	Reaction	Reference
Lipid	Sudan IV	+	Sorokin (1958)
	Nile Blue	++	Jensen (1961)
	Polychrome Methylene Blue	+	Van Fleet (1950)
	Ruthenium Red	+	Sorokin (1958)
Protein	Mercuric Bromphenol Blue	-	Mazia et al. (1953)
	Picric Acid	-	Johansen (1940)
	Aqueous Eosin	-	Johansen (1940)
	Biuret Test	-	West and Todd (1962)
Callose	Aniline Blue	-	Currier (1957)
Carbohydrate	Fehlings	?	Sass (1940)
	Benedicts	?	Sass (1940)
	Tollens	++	Cormack (1948)

Key:

-	negative reaction
+	very slight positive reaction
++	positive reaction
?	color change but difficult to assess

the material due to the action of the propylene glycol was noticed in every section (see fig. 47). The cross striations marked in fig. 47 are similar in appearance to those shown by Sorokin (1958), when sections of pea stem were mounted in this reagent. The most striking lipid staining reaction is shown with Nile Blue. A 1% aqueous solution of this dye at 37° C. and then cleared with 1% acetic acid, stains the intercellular material a brilliant red. Figures 45 and 46 show the appearance of the material in longitudinal view after being stained with Nile Blue. Jensen (1962), claims that Nile Blue imparts a red color to fats, oils and waxes and a blue color to free fatty acids and phospholipids. The red staining reaction of the intercellular material in the fresh sections suggests that the constituent lipids are not free fatty acids or phospholipids. Polychrome Methylene Blue (0.25 g. dye, 4.0 g. lithium carbonate in 300 ml. water heated to 90° C. for 10 minutes, and 5 ml. glacial acetic acid) added to fresh longitudinal sections stains the material a slight pink-purple color. Van Fleet (1950), states that a red-lavender color is indicative of neutral fats while a blue color indicates free fatty acids.

When sections are perfused with a 0.01% aqueous solution of Ruthenium Red, a very slight red color is produced in some of the intercellular material. Although this dye generally stains pectic material, it is not specific, as nuclei, some lipids and polysaccharides may give the same

PLATE VIII.

(All photomicrographs are of living white mustard root tips which were cut free hand with a sharp razor blade.)

Figure 44. (X600) Typical reaction of the intercellular material to H_2SO_4. Odd-shaped structures form which finally round-up into spherical bubbles. The disintegrating cells can be seen in the background.

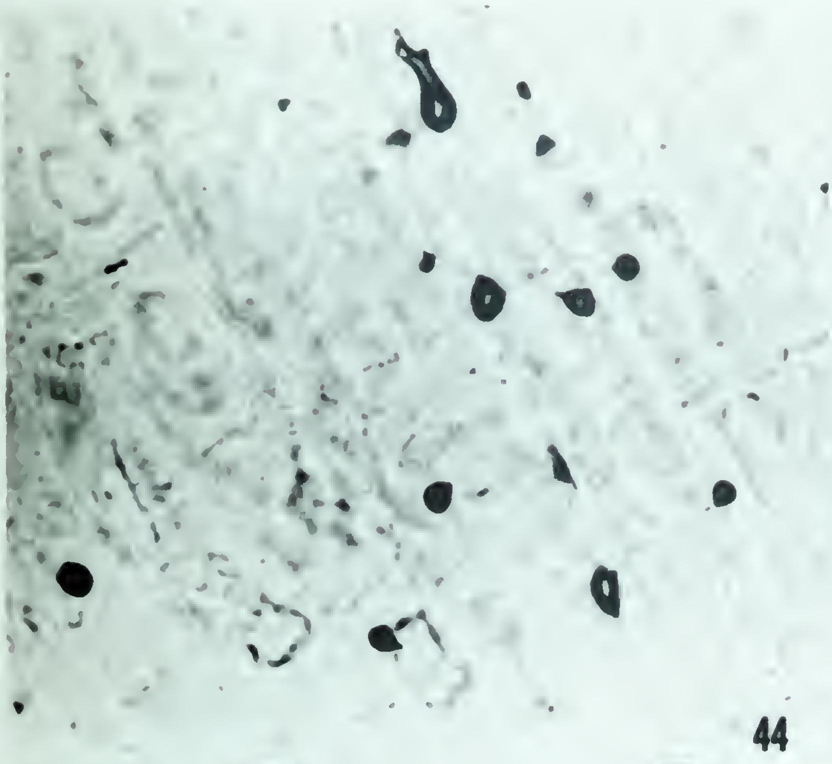
Figure 45. (X500) A free-hand longitudinal section of the root tip showing a tubular structure which stains positively with Nile Blue.

Figure 46. (X830) An enlarged view of Figure 45. The tube to the right shows longitudinal striations running the length of the tube.

Figure 47. (X2050) Typical reaction of the material to propylene glycol. The material swells, and in longitudinal view, cross striations (indicated by the arrow) become evident.

Figure 48. (X950) Cortical spaces near the meristem in the primary root of _Zea_ _mays_. Black particles lining the spaces become evident shortly after Tollen's reagent has been added.

Figure 49. (X2500) A section of the same root shown in Figure 48 showing two enlarged intercellular spaces.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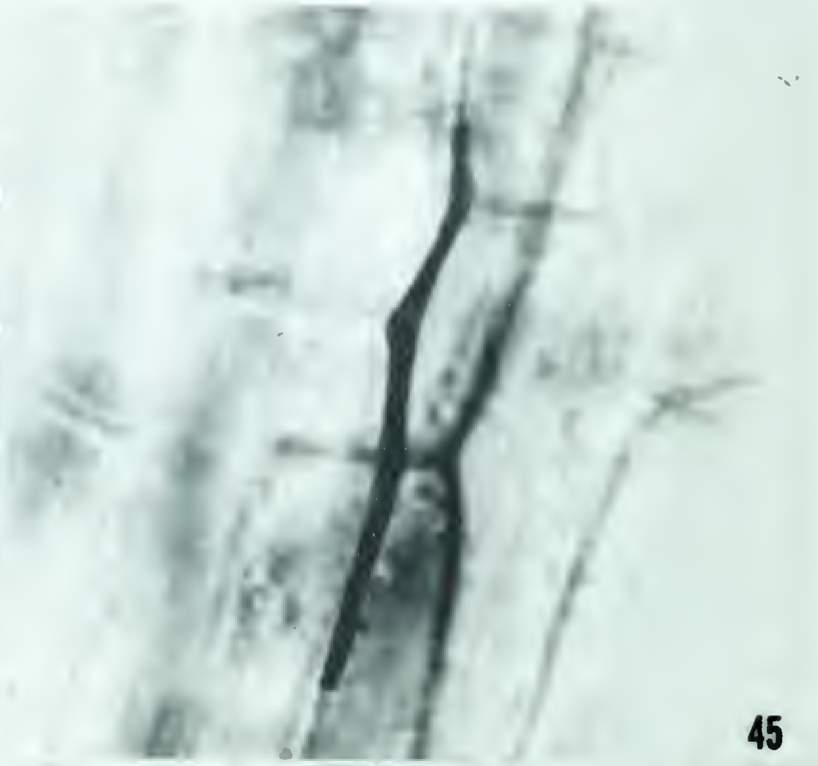
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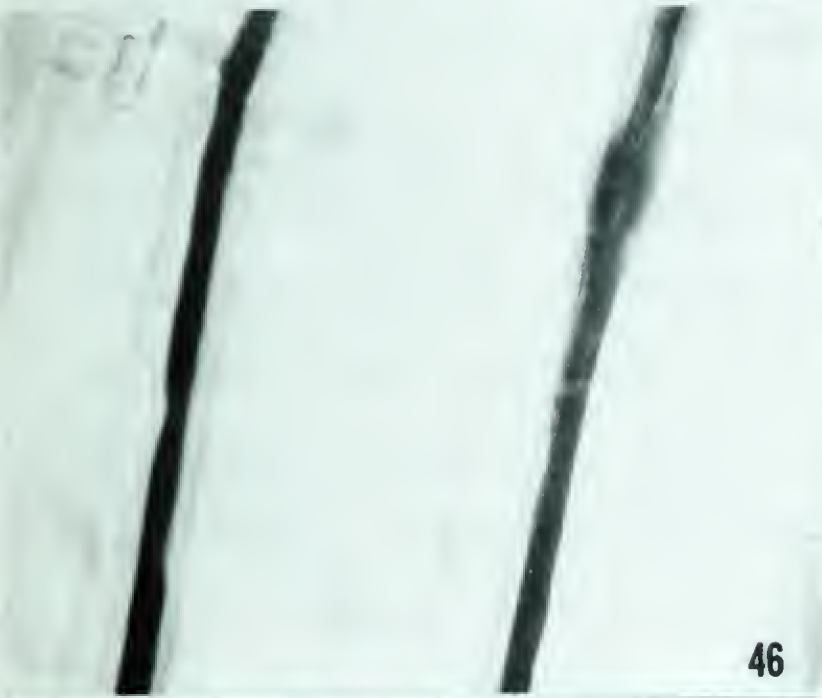
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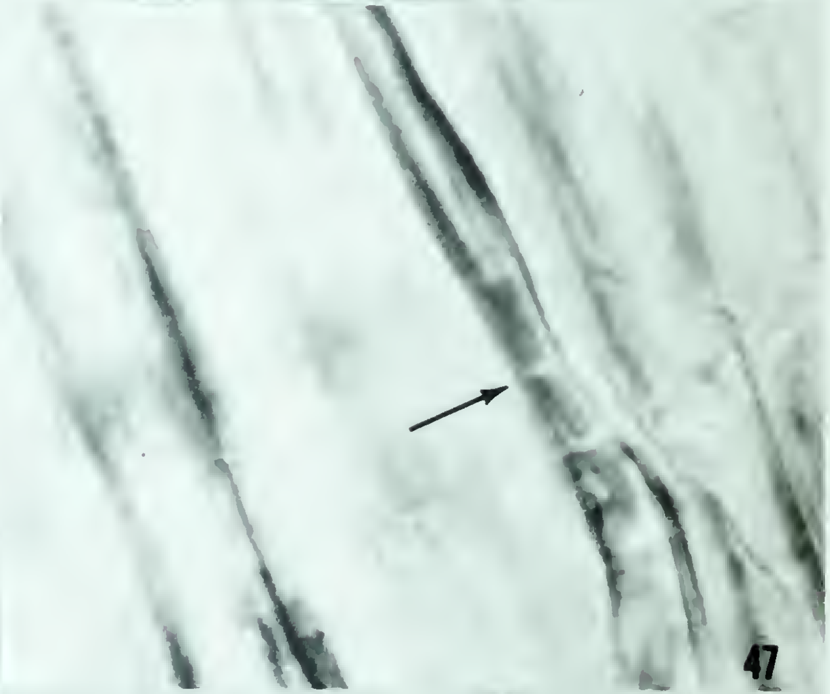
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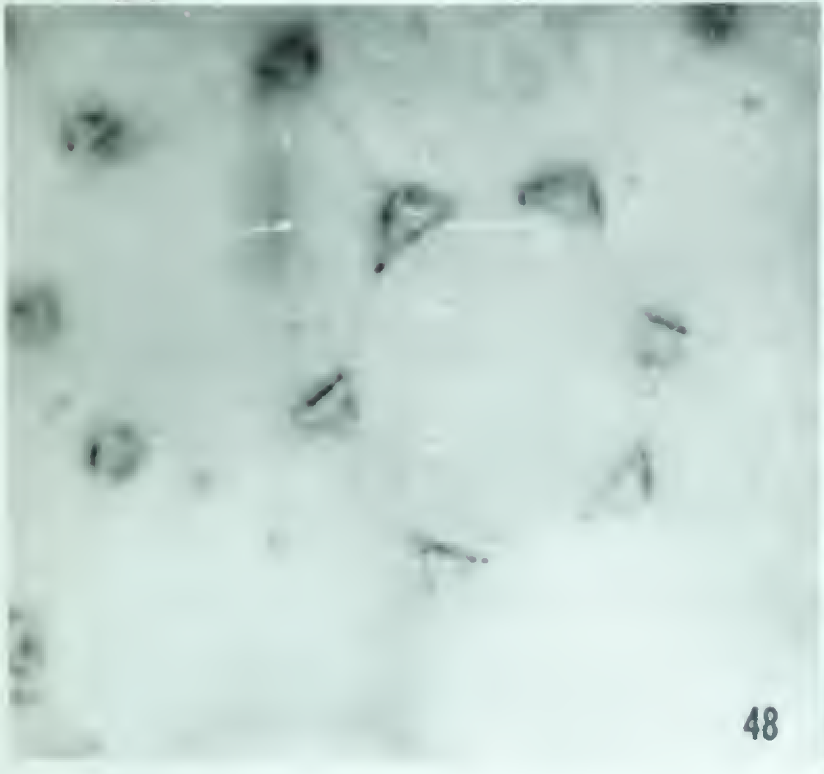
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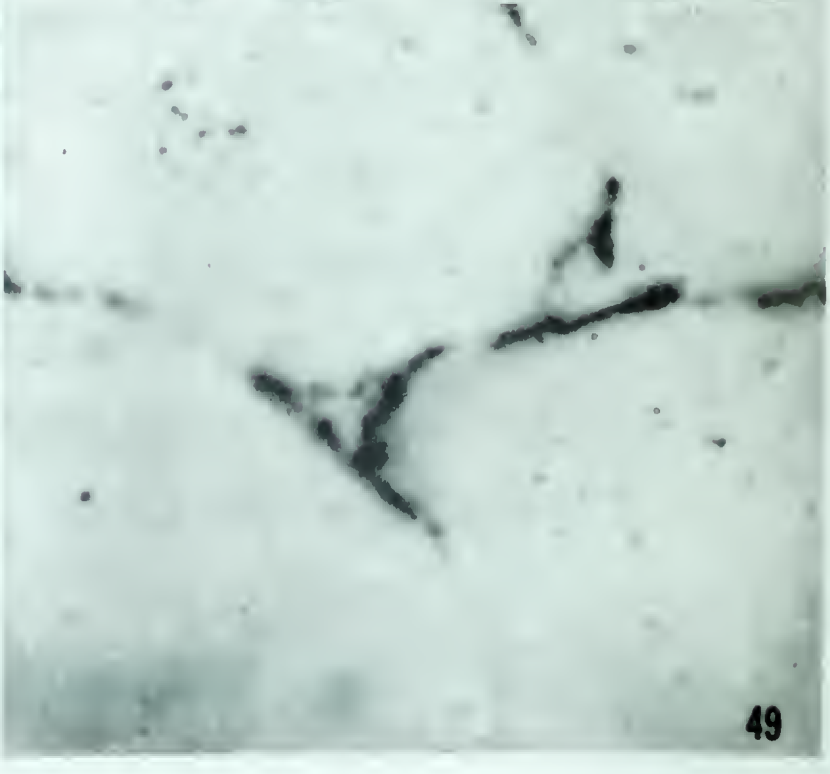
49

reaction (Sorokin, 1958).

The possibility that the enclosing membrane of the intercellular material is of a lipoprotein nature was investigated using a number of protein-specific stains. Preparations immersed for 15 minutes in a 0.10% aqueous Mercuric Bromphenol Blue solution (a general protein stain) and then washed for 3 minutes in distilled water, show the original dark color in the intercellular spaces. To test the specificity of the dye for protein, drops of the dye were added to gelatin in a spot plate. An immediate brilliant blue was produced in the gelatin. Further test for protein included the Biuret test, and staining with a 0.001% aqueous solution of Eosin and with a saturated aqueous solution of picric acid. All the tests were negative (see Table VIII).

A demonstration of the presence of sugars was attempted in a number of ways. Cross sections of the root were placed directly into either Fehling's or Benedicts' reagent on a slide and brought to boiling point three or more times with the addition of more reagent when necessary. Immediate microscopic examination showed a yellow color in the partially disintegrating cortical tissue and the original dark color in the intercellular spaces. With an increase in light intensity and by focusing up and down, a slight orange-brown color was apparent in the intercellular material. A second method consisted of placing three drops of the test reagent at separate locations on a microscope slide, allowing it to

dry slightly, and adding a fresh transverse section of the root to each of the partially-dried drops. The controls were identical but a drop of a 1% solution of glucose replaced the root sections. All the slides were warmed slightly and then set aside at room temperature for periods ranging from 24 hours to several days. The drops on the control slides became orange-brown within approximately 48 hours. To examine the test slides, they were inverted on the microscope stage and viewed through the bottom of the slide. All the sections were dessicated and yellow in color. Dark areas corresponding with the former location of the intercellular spaces could be noticed in most sections. With high light intensity, a slight orange-brown color was evident in some of the intercellular spaces.

A microchemical technique designed to detect the presence of a reducing substance in the spaces of white mustard roots (Cormack, 1948), was modified and used in the present study. Tollen's reagent prepared by adding concentrated ammonium hydroxide to a 3% solution of silver nitrate until the precipitate that formed just redissolved, was used but instead of allowing intact roots to react for approximately 18 hours, fresh transverse sections were mounted on a microscope slide and the test reagent quickly drawn under the cover glass during continuous microscopic examination. The intercellular spaces containing the dark intercellular material become blacker than usual but no other effect on the material

is noticed. Some spaces near the apex that are apparently devoid of contents now have dark-staining spherical bodies adhering to the walls forming the spaces. Sections of Zea mays treated in the same way give similar results. Figures 48 and 49 are typical of results obtained with both corn and white mustard roots. There is little doubt of the authenticity of these results as they are easily reproduceable. The possibility that substances escape into the spaces as the cells are cut is unlikely since some of the spaces in the same region fail to show a reaction and spaces in the mature regions of the root remain clear.

The widespread occurrence of callose throughout the plant and its possible function as an intermediate in the degradation and synthesis of cellulose (Currier, 1957), suggested the possibility of it being a constituent of the intercellular material. A 0.005% aqueous solution of Aniline Blue was added to sections during continuous microscopic examination. In all sections, the intercellular material remained black.

v) Effects of lipase

The effects of lipase on the intercellular material were tested by adding fresh transverse sections of the root tip to a solution of the enzyme either on a spot plate or on a depression slide. A 1% solution of the enzyme was prepared by adding either Lipase 448 (Nutritional Biochemicals

Corporation, Cleveland) or Lipase (Steapsin) to a phosphate buffer containing a few drops of 0.05% $CaCl_2$. Solutions with pH's ranging from 7.0 - 8.6 were prepared. Sections were either observed continuously under the microscope or were incubated in covered spot plates at 37° C. for periods up to 24 hours before examination. The enzymes used for the tests were shown to be active by their rapid breakdown of olive oil to fatty acids, as indicated by the contrast in color reaction with Nile Blue in control samples as compared to those with the added enzyme. The lipase-treated olive oil stained a deep blue as compared to a light red in the controls. In no case was there a noticeable change in the appearance of the intercellular material when fresh sections were treated with lipase. The failure to produce a change in the material with this enzyme may be due to the small amount of lipid present or to the inhibition of lipase by substances escaping from the cut cells.

DISCUSSION

A. Tissue Differentiation and Maturation under Normal Conditions

Although no systematic attempt had previously been made to study the complete development of all primary root tissues in white mustard, various recent workers have followed the development of one or two specific tissues.

The results of the present investigation clearly support the conclusions of Goodwin and Stepka (1945), and Popham (1955), that the demarcation of the root tip into four definite zones, each associated with a single developmental process does not accurately describe the true pattern of development in growing root tips.

The inferences which can be made from the present series of observations are as follows. First, each tissue differentiates and matures at its own rate and not within a particular region common to all tissues of the root. At one specific level, one kind of tissue may be almost mature, another still differentiating, and another tissue undergoing cell division. For example, at approximately 500 μ's from the root apex, a sieve tube is mature at both protophloem poles while the cells of the protoxylem have differentiated but are not mature, and cells near the periphery of the vascular cylinder are still dividing. Second, the level of differentiation of certain tissues is strikingly constant from root to root while that of other tissues varies consider-

ably. For example, the level of first appearance of short and long cells of the epidermis, and the differentiation of the pericycle, endodermis, protoxylem, and metaxylem is very consistent. Similarly, the level at which one, and then two sieve tubes of the protophloem differentiate and then mature varies within a narrow range. On the other hand, the appearance of root-hair papillae, the extent of the root cap basally from the cap initials, and the vacuolation of epidermal and cortical cells, all show a slightly wider range of variation. The variation of the vacuolation measurements is easily understood since the criterion used is necessarily subjective. Cormack (1949), and others have indicated that environment plays an extremely important role in root-hair formation and therefore the variation in the appearance of the first papillae is to be expected. More variable still are the ranges for lignification and subsequent maturation of the protoxylem. From the data of other investigations (*e.g.* Heimsch, 1951; Popham, 1955), this seems to be a feature common to other roots.

Third, the sequence of events of differentiation and maturation of primary tissues in the root tip of white mustard as shown in fig. 28, agrees with that reported for a few other dicotyledonous roots (Esau, 1938; Popham, 1955). Future detailed examinations of other plant species should assist in the formulation of a general picture of differentiation and maturation in root tips, which would be suitable

for presentation to students in elementary botany courses.

B. Tissue Differentiation and Maturation in Experimental Environments

Although an inhibition in growth rate of the primary root generally results in an acceleration in differentiation and maturation of tissues (Bryant, 1934; Esau, 1943b, 1954; Heimsch, 1951; Popham, 1955; Torrey, 1953; Wilcox, 1962), this generalization is not without exception. Data obtained in the present study confirm the generalization, but the levels are not always nearer the apex in slow-growing roots or furthest from the apex in the fastest growing roots. Furthermore, the values obtained for levels of differentiation of most of the tissues in the experimental environments are only slightly different. With the exception of the lignification and maturation of the protoxylem and the appearance of root-hair papillae, marked differences in differentiation and maturation rates with altered environmental conditions do not occur. Growth of roots in a moist-air environment at varying temperatures results in the least modification in rates of differentiation and maturation as compared to those grown under normal conditions.

Popham (1955), suggests that levels of differentiation and maturation in *Pisum sativum* primary roots are not determined by environmental conditions, root length, or root age, but are strongly controlled by genes. The apparent environ-

mental control of root-hair development is cited as an exception to this genic control concept. Results of the present study suggest a strong genetic control of differentiation of tissues in the primary root of white mustard.

A possible explanation for the early formation of lignified secondary walls in the protoxylem cells in slow-growing roots as compared to fast-growing roots is offered by Bryant (1934). The concentration of reducing and total sugars of barley roots grown in aerated culture is less than in those grown in non-aerated cultures. (Similar results were obtained by van der Heide _et al_. (1964)). The greater amount of oxygen available to the roots, with its accelerating effect on the respiratory rate, decreases the sugar content to a point where it becomes the limiting factor in cell wall thickening. Recent work, (Wetmore and Rier, 1963), has shown that vascular tissue formation in callus tissue can be induced by a critical balance between auxin and sugar concentration. It is probable that future experimentation will show that the level of xylem lignification can be varied according to the sugar and auxin content of the tissues.

In conclusion, it seems reasonable to propose that the levels of differentiation of primary tissues in white mustard root tips are affected only slightly by the experimental environments used in the present study, while levels of protoxylem lignification and maturation and the development of root

hairs are more directly modified.

C. Intercellular Spaces and their Content

The many observations of fresh sections of white mustard root tips made in the present study lead to the conclusion that intercellular spaces close to the apex contain a substance which is not entirely a gas.

The similarity in size and shape of intercellular spaces in roots grown under reduced pressure and those grown under atmospheric pressure, might be explained by the presence of such a material, but the possibility that the gas occupying the small spaces may not be of sufficient volume to expand appreciably can not be overlooked.

The characteristic distribution pattern of the material in the root suggests that it is being actively removed from the spaces in some way. Sorokin (personal communication) states, "... you can find it in any part of the plant where cells are enlarging and elongating. Therefore it is my strong conviction that this material represents a substance which contributes in one way or another to the formation of the cell wall." Although the present study adds little to our knowledge of the function of the intercellular material, further evidence is added as to the existence of such a substance, and results obtained by treating fresh sections with various chemicals and biological stains indicate some of its physical and chemical properties. The invariable

dissolution of the substance in lipid solvents and the positive reaction for lipids with some stains indicates that lipids are probably constituents of this material. The dissolution of the material in the organic solvents is indisputable, but the staining reactions are far from conclusive. An obvious difficulty is the choice of stains or histological tests that are specific and yet are either soluble in a solvent that does not affect the material, or require mild conditions so that cell detail in the fresh sections is not lost. Because of this, many common or well-known specific tests could not be used. A method to overcome this difficulty was tried without success. Fresh transverse sections were added to a small amount of petroleum ether on a microscope slide, allowed to sit for a few seconds, and then removed. The petroleum ether was collected with a fine pipette and kept in micro test tubes. A number of test tubes were filled in the same manner in preparation for testing with various stains and chemical reactions. None of the tests showed positive results probably due to the small amount of material that could be collected in this way.

The possibility of using gas chromatography to identify the chemical nature of the material was discussed but after the difficulty experienced in extracting this material and the possibility that substances occurring in the chromatogram could also come from the cells injured during cutting, the idea was not followed through.

That the existence of such an intercellular material has been overlooked for decades is understandable, since the material undergoes rapid dissolution in reagents commonly used in fixing and dehydrating plant tissue for subsequent embedding in paraffin.

One of the most interesting observations in the study of intercellular spaces is the effect of Tollen's reagent on fresh tissue sections of the primary root of white mustard and Zea mays. This reagent can be reduced by a number of organic substances that are easily oxidized. The reaction observed in the present study was identical to that observed when intact primary roots of white mustard were treated for a number of hours in the same reagent (Cormack, 1948). Black spherical bodies lining the intercellular spaces were observed in both cases. In the present study, this reaction is shown only by the spaces close to the meristem that lack the dark intercellular material. Since Cormack and Lemay (1963), have shown that sugars can be translocated from the cotyledons through the intercellular space system of the roots, and since certain sugars containing a free aldehyde or ketone group will reduce Tollen's reagent, it seems likely that the reaction is due to reducing sugars.

The major conclusion reached from the present study of intercellular spaces and their content is that a substance other than a gas is present in some of the spaces in the primary root of white mustard. There is some evidence that

lipids may be constituents of this material but further experimentation is required to determine its exact chemical nature and function in the growing root tip. This study also provides more evidence for the possible presence of a reducing sugar in the youngest spaces.

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APPENDIX I

Primary root length (mm.) in a 24 hour growing period after germination. Measurements represent a random selection of 20 roots.

	Experimental Environment		
	Non-aerated Water	Aerated Water	Moist Air
	9.5	12.0	21.5
	5.0	12.0	21.0
	4.5	10.5	11.0
	5.5	9.0	22.5
	5.0	11.5	20.0
	6.0	11.0	23.0
	5.0	8.0	20.5
	5.0	10.0	15.0
	5.0	7.5	14.0
	5.0	8.5	15.0
	5.5	10.0	17.0
	6.0	9.0	19.0
	5.0	10.5	15.0
	4.5	11.5	19.0
	5.5	8.5	15.0
	6.5	10.0	17.0
	5.0	9.5	19.5
	6.0	9.0	18.0
	5.0	8.0	13.0
	5.0	10.0	16.0
MEAN	5.5	9.8	17.6